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INFLUENCE OF AGE OF MOTHER ON APPEARANCE OF AN HEREDITARY VARIATION IN *HABROBRACON*.

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Contentions about the inheritance of acquired characters have frequently involved questions of the age of the parents. Thus in trotting horses older sires or dams which have undergone considerable training have been supposed to transmit some of the benefits of this training to their offspring. Similarly, acquired knowledge, acquired immunity to disease, etc., have been stated to be transmitted. When these matters are investigated scientifically it is found that unintentional selection of parents may account for the bulk of cases. Nor is character of offspring which may be found to be correlated with parental age to be taken as proof of change in germ plasm of parents. Some non-germinal influence may be transmitted. Thus it has been shown (Hart, 1923) that Mongolian idiots are at their birth twenty-three times as likely to have mothers forty years of age or over as mothers from twenty to twenty-four. There is no good evidence that Mongolian idiocy is hereditary.

The material considered in the present paper presents clear evidence of change in average traits of offspring according to age of mothers. Discussion as to whether this difference is germinal will be reserved for a later part of the paper.

In the parasitic wasp *Habrobracon juglandis* (Ashmead) which has been bred under constant environmental conditions, abnormalities in antennæ, external genitalia or posterior part of the digestive tract occur.

Abnormal antennæ may be branched, bent or clubbed.

Gonapophyses of the female (sensory appendages forming sheath for the sting) may be swollen at the end or twisted from

their normal position; extra gonapophyses may appear in addition to the two normally present.

These abnormalities which consist in increase in size or number of parts or in malformation without reduction are to be distinguished from *deficiency*.

Deficiency.

Antennal deficiency is usually evidenced by tapering and fusion of terminal joints as in right antenna of specimen shown in Plate 1, Fig. *H* (Male from 1171.3*c*). There is, in general, a tendency toward symmetry but much irregularity. Fusion without tapering may occur or one antenna may be normal (Figs. *A* and *H*).

Males may have genitalia missing and abdomen rounded off at tip, or the normal parts may be reduced in size, twisted out of shape or lacking on one side of the body while present in almost normal condition on the other.

In Plate I., Fig. *D* is shown the ventral side of the abdomen of a normal male. Fig. *E* shows the abdomen from posterior view, somewhat oblique, with genitalia extruded. The tip is curved ventrally so that it is seen in dorsal aspect.

Figs. *A*, *B*, and *C* are from a male (Freak 60) illustrating deficiency in antennæ and genitalia on the right side of the body both anteriorly and posteriorly. Fig. *B* shows the tip of the abdomen ventrally and slightly oblique while Fig. *C* is an aspect comparable with Fig. *E*. Genitalia are completely lacking on right side, including even the right side of the penis, while posterior sternites and tergites taper off dextrally. Organs on the left are somewhat bent toward the right but otherwise normal. Sections of abdomen showed right testis and vas deferens completely lacking, left apparently normal.

Fig. *A*, ventral view of head, shows left antenna normal. It is drawn displaced from the head for economy of space. Right antenna is shortened by reduction and fusion of joints toward the tip. Right labial palpus is missing.

Figs. *K*, *L*, and *M*, show dorsal, ventral, and right aspect respectively of abdomen of normal female.

Figs. *I* and *J* show ventral and right aspect respectively of

abdomen of a deficient female (Freak 6). Great reduction and fusion of parts posteriorly, especially of gonapophyses, is to be noted. Sections show large eggs and nurse cells developing, but no trace of poison apparatus; deficiency therefore extended internally to posterior organs.

Reduction in the posterior part of the digestive tract also occurs. This may be indicated by a swollen appearance of the abdomen with an abnormally dark color, due to failure of elimination of fecal matter. Sections show different degrees of defect ranging from an apparent failure of the proctenteron to connect with the mid-gut, to a complete disappearance of the former and considerable reduction of the latter posteriorly. This reduction may shorten the Malpighian tubules until they are little knobs on the posterior end of the mid-gut sack, or the latter may be rounded off smoothly. Fig. *F* shows a specimen of this type from a female (Freak 21) with no external genitalia. There were no hind-gut, no Malpighian tubules, no poison apparatus. In the ovaries nurse cells were present but ova were small. The posterior compound nerve ganglion was normal.

Fig. *G* is from a male (Freak 20) with no external genitalia, and no hind-gut posterior to the Malpighian tubules which are short and thick. The mid-gut is much swollen with fecal matter. Testes were present, filled with mature sperm but there were no vasa deferentia. The posterior compound nerve ganglion was separated into its three elements.

Rare Occurrence of Abnormalities in Normal Stocks.

Among 53,148 males and 16,036 females of normal stocks the following abnormalities in antennæ, genitalia, and digestive tract have appeared.

Only twelve females had abnormal gonapophyses.

Abnormal antennæ were recorded in only thirty males and twenty-six females. These may be classified as having one or more antennæ short, sixteen; absent, twenty-eight; clubbed, one; branched, three; and unclassified, eight. Two of those having short antennæ were males with deficient genitalia.

Association between deficiency in genitalia and digestive tract is much closer. Males deficient in genitalia alone were twenty-

four; in digestive tract alone, fifty-five; and in genitalia and digestive tract, fifteen. Females deficient in genitalia alone were nine; in digestive tract alone, twenty-two; and in genitalia and digestive tract, nine.

These figures illustrate the rare occurrence of these abnormalities in normal stocks.

Deficiencies in genitalia necessarily entail sterility. Mating reactions are in all cases quite normal. A male having no trace of external genitalia and internal much reduced will mount a female and beat its antennæ in the usual manner. Females with reduced genitalia or abdomen swollen and blackened by fecal matter show characteristic responses to caterpillars. Although wasps with deficient digestive tract live only a few days and females lay but few eggs or none it has sometimes been possible to obtain offspring from them. These offspring are usually normal.

Strain Showing Hereditary Deficiency in Genitalia and Digestive Tract.

Hereditary deficiency in genitalia and in digestive tract arose from the combination of material from Lancaster, Pennsylvania, and Iowa City, Iowa. No deficient occurred in the direct line of Iowa City ancestry which included 598 males and 543 females running back to the original female. An I male was crossed to an L female, stock 6. An F_2 male from this cross was mated to an L female, stock 10. This resulted in fifty-three normal males and 130 normal females, besides one female with deficient digestive tract. This is the first occurrence of the character in the ancestry. A sister of this female produced 224 normal males one of which was mated to an I female. Fifty normal males and ninety-six normal females resulted. One of these females produced 139 normal males, five deficient males and one impaternate female. (Female from unfertilized egg—from 934.5.)

Descendents of this impaternate female include in all 209 fraternities. Inbreeding was carried on by simply isolating individual females after they had had a chance to mate with their brothers. Ratios of deficients in these fraternities varied erratically and a large number of insects died immature. The latter were recorded without reference to sex.

Ratios of deficients among the males in the 209 fraternities were arranged in a frequency distribution. Grouped to the nearest 2 per cent. they ranged from 0 to 58 per cent. with frequencies 57, 6, 4, 9, 11, 21, 19, 8, 6, 5, 7, 5, 3, 8, 6, 6, 5, 5, 0, 6, 2, 0, 0, 2, 0, 2, 0, 1, 1, 2, besides two fraternities each consisting of a single deficient individual.

131 of these fraternities included females with ratios of deficient ranging from 0 to 34 per cent. Corresponding frequencies were 80, 1, 10, 9, 7, 2, 4, 7, 2, 2, 1, 0, 0, 3, 1, 0, 0, 2. There were also two small fraternities, one with one deficient to one normal and the other with four deficient to two normal.

Percentage of wasps dying immaturely were calculated for the 209 different fraternities. These ranged from 0 to 32 with frequencies 125, 11, 17, 19, 12, 5, 6, 2, 3, 1, 3, 0, 1, 1, 2, 0, 1.

There were 10,335 males and 2,165 females in the entire group.

Frequency According to Age of Mother.

In a special line of this deficient stock consisting of thirty-seven cultures there were 2,094 males with 540 or 25.788 per cent. deficient, 468 females with 43 or 9.188 per cent. deficient, and among the total of 2,732 there were 170 or 6.223 per cent. dying immature.

Females are transferred every four or five days and culture vials labelled *a*, *b*, *c*, etc. Thus if offspring be summarized according to letter of vial average changes in progeny according to age of mothers may be obtained. Table *A* and Chart 1 show

TABLE *A*.

OFFSPRING OF STRAIN DEFICIENT IN GENITALIA AND DIGESTIVE TRACT ARRANGED ACCORDING TO AGE OF MOTHER (VIALS).

	Vial.							
	a	*b*	*c*	*d*	*e*	*f*	*g*	Total.
Males, Total.....	694	458	370	318	148	70	36	2,094
Deficient.....	109	135	129	120	34	10	3	540
Females, Total.....	168	196	96	8				468
Deficient.....	12	17	14	0				43
Total.....	888	706	513	358	160	71	36	2,732
Immature deaths...	26	52	47	32	12	1	0	170

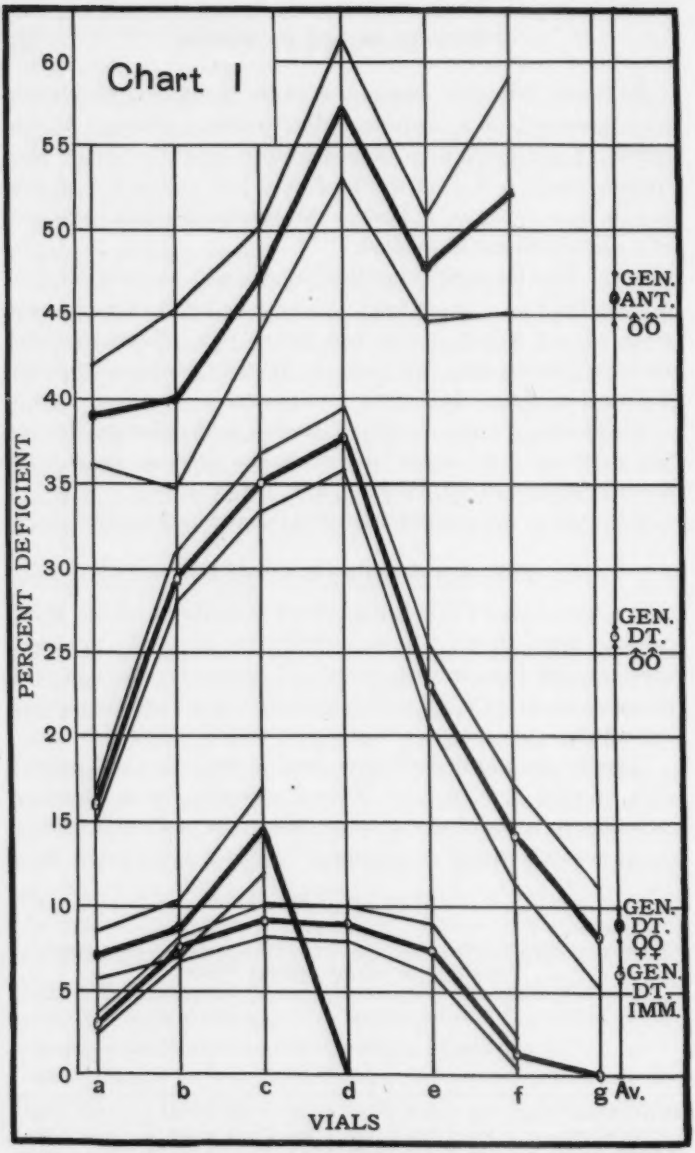

CHART 1. Percentage of deficient offspring according to age of mothers. Culture vials through which mothers were successively transferred are denoted by *a*, *b*, *c*, *d*, *e*, *f*, *g*. Mothers remained in each vial between four and five days. *Gen. Ant.* and *Gen. Dt.* denote offspring from mothers of strains in which deficiency in genitalia was associated with deficiency in antennæ and in digestive tract respectively. For actual numbers see Tables *A* and *D*.

TABLE *B*.

OFFSPRING DEFICIENT IN GENITALIA OR DIGESTIVE TRACT ARRANGED ACCORDING TO TYPE OF DEFICIENCY AND AGE OF MOTHER (VIALS).

	Vial.							
	a	*b*	*c*	*d*	*e*	*f*	*g*	Total.
Males								
(Genitalia reduced): actual	6	10	7	5	1	0	0	29
per cent	5.5	7.4	5.4	4.2	2.9	0	0	5.37
(Genitalia absent): actual	74	100	79	85	22	7	3	370
per cent	67.9	74.1	61.2	70.8	64.7	70.0	100.0	68.52
(Deficient digestive tract): actual	4	0	5	5	1	1	0	16
per cent	3.7	0	3.9	4.2	2.9	10.0	0	2.96
(Deficient genitalia and digestive tract): actual	25	25	38	25	10	2	0	125
per cent	22.9	18.5	29.0	20.8	29.4	20.0	0	23.14
Females								
(Deficient genitalia): actual	2	3	1					6
per cent	16.6	17.6	7.1					13.95
(Deficient digestive tract): actual	8	13	11					32
per cent	66.6	76.4	78.6					74.42
(Deficient genitalia and digestive tract): actual	2	1	2					5
per cent	16.6	5.8	14.3					11.63

results of such summarizing for this group. It may be noted that tendency toward production of deficiency is relatively low when mothers are young but increases up to the tenth to fourteenth day when it rapidly falls. Essentially the same form of curve is seen for deficient males, deficient females, and immature deaths but data for females are rather meager.

The various types of deficients as they occurred in the different bottles are recorded in Table *B*.

By glancing at the percentages in this table it may be seen that there is no consistent and significant change in ratio of any type of deficiency according to the age of the mother. The continuous increase in females with deficient digestive tract is not to regarded as significant because of small numbers.

Crosses of Deficient and Normal Stocks.

All females of the deficient line were lost through their failure to produce daughters. Their sons were crossed with normal L stocks. Daughters were back-crossed to the same males for three generations. Although the males came from the selected material and were used as parents for four successive generations, the ratio of deficient did not exceed two per cent. 6,954 wasps of the third generation were bred. Wasps were deficient in genitalia alone, ninety-six; in digestive tract alone, twenty; in genitalia and digestive tract, fourteen.

A male from the selected deficient line was crossed to a female from Iowa City stock 11. The male had orange eyes, wrinkled wings, defective r_4 vein, and yellow mesosternum. Stock 11 is characterized by black eyes, flat wings, normal r_4 vein and sooty mesosternum. Orange acts as a unit with no overlapping, wrinkled has but little. Defective and sooty are variable multiple factor characters in this cross.

The deficient in F_2 numbered among 2,252 males only thirty-five or 1.55 per cent., and among 957 females only five or 0.52 per cent.

The thirty-five males included nineteen orange, fifteen wrinkled, and twenty sooty. Among the twenty in which venation could be determined ten had defective r_4. There is thus no association between deficiency and the characters mentioned.

These males were classified as genitalia aberrant, one; genitalia lacking, nineteen; digestive tract deficient, nine. Four females were deficient in digestive tract, one in genitalia and digestive tract.

Strain Showing Hereditary Deficiency in Genitalia and Antennæ.

Deficiency in genitalia and in antennæ characterized cultures derived from a female of Lancaster stock 10. Deficiency in digestive tract did not occur among these with the exception of a single male (of fraternity F_1A in Table *C*). This specimen was also deficient in genitalia and antennæ.

Table *C*.

Males Showing Deficiency in Antennæ and Genitalia. Three Generations Descended from "Mutant" Female from Stock 10.

Fraternities.	Total.	Deficient.			Total Deficient.	Per Cent. Deficient.
		Genitalia.	Antennæ.	Genitalia and Antennæ.		
F_1A.....	104	6	4	21	47*	45.2
F_2A.....	3				0	0.0
B.....	9				0	0.0
C.....	22				0	0.0
D.....	72		1		1	1.4
E.....	115		2		2	1.7
F.....	39		1		1	2.6
G.....	66	3	2		5	7.6
H.....	10		1		1	10.0
I.....	12			4	4	33.3
J.....	30	2	3	8	13	43.3
K.....	76		4	30	34	44.7
L.....	112	8	1	47	56	50.0
F_3A.....	81				0	0.0
B.....	88	1	2		3	3.4
C.....	151		6	62	68	45.0
Total..... F_1A..... F_2I–L..... F_1C.....	485	16	18	172	222*	45.8
Total..... F_2A–H..... F_2A–B.....	505	4	9		13	2.6

* Sixteen unclassified deficient of vial *a* are included here.

The stock 10 female was isolated as a virgin August 1924. After passing through vials *a*, *b*, and *c* she was mated to her sons from *a*. Daughters appeared in the succeeding bottles. Her progeny totalled 104 males, twenty females, and one immature.

All the females were set. Eight proved sterile and twelve fertile. In later generations female sterility also appeared. In the total line there were seventy-two females set of which twenty-one failed to produce offspring although they lived the average length of time.

There were 473 females in the whole line of which one had deficient genitalia, one had tapering antennæ and one showed both defects.

There were recorded only four immature deaths.

Males of F_1, F_2, and F_3 from one F_2 fraternity are recorded in Table *C*. Other F_3 males and males of later generations consisted of 489 normal and eight deficient. These eight occurred in F_3 after which no more appeared.

The fraternities of males recorded in Table *C* evidently fall into at least two groups.

The six fraternities F_1A, F_2I–L, and F_3C are the only ones showing males with deficiency both in genitalia and in antennæ. They are highly deficient, the ratio averaging 45.8 per cent.

Deficiencies in antennæ and in genitalia are highly correlated in this group for they both occur in 172 cases out of 206. (Vial *a* of the F_1 generation could not be included in this total as the deficient were not separated into the three groups.) The remaining ten fraternities of Table *C*, F_2A–H, F_3A–B, with 505 males have only thirteen or 2.6 per cent. deficient. None of these is deficient in both characters.

Frequency According to Age of Mother.

The males of the six fraternities showing High Deficiency have been summarized with respect to vials in order to test influence of age of mother on ratio of deficient offspring. Table *D* gives results of this summary and Chart 1 shows percentage frequencies.

The rise from *a* to *d* is, as in the case of deficiency in genitalia, associated with deficiency in digestive tract, clearly significant. The analogous drop to *e* is of doubtful significance; the subsequent rise of no significance.

TABLE *D*.

MALES OF STRAIN DEFICIENT IN GENITALIA AND ANTENNÆ ARRANGED ACCORDING TO AGE OF MOTHER (VIALS).

	Vial.						
	a	*b*	*c*	*d*	*e*	*f*	Total.
Total	116	40	123	72	111	23	485
Deficient	45	16	58	38	53	12	222

It may be concluded that age of mother is an effective factor in determining this type of deficiency.

DISCUSSION AND SUMMARY.

Deficiency or reduction in antennæ, in external genitalia, and in posterior part of the digestive tract occurs occasionally in individuals of normal stocks.

Two cases of marked hereditary deficiency present some interesting points of difference. In one, deficiency in genitalia was associated with deficiency in digestive tract and many immature deaths occurred. The trait was increased and maintained by selection but very few fraternities approximated 50 per cent. even among the males. Great variability in ratios in the different fraternities and failure to recover the stock by breeding normal up to males of selected deficient indicate that the character is genetically complex.

In the other case, deficiency in genitalia was associated with deficient antennæ. There was no increase in immature deaths over those normally occurring but much female sterility. The line originated from a female from stock supposedly normal. Fraternities fell into at least two groups. In one the deficient averaged 2.6 per cent. of the total and were deficient either in genitalia or in antennæ but not in both. In the other they averaged 45.8 per cent. of the total, the majority of them being deficient both in antennæ and in genitalia. The facts indicate genetic complexity.

Percentage of deficient offspring varies according to age of the mother in both cases. It increases up to approximately the tenth

or fourteenth day of adult life and then decreases. No consistent change in type of deficiency according to mother's age could be detected.

Possible explanations of this rise and subsequent fall of ratio of deficient during life of mother may be considered.

Cytoplasm or yolk of eggs might differ according to some rhythm in the life of the female. The influence would have to be carried through the larval period and up to the time of the formation of the organs in the pupa.

Something in the food of the larva might vary according to the age of the mother, some ingredient of the fluid injected at time of stinging the caterpillars.

Neither of these theories involves genetic change in maternal contribution.

A third explanation may be suggested. It has been shown that linkage in *Drosophila* varies with age of mother. If this is true in *Habrobracon* a difference in genetic composition of offspring according to the mother's age may be brought about. In order to test this possibility records on a lethal factor linked with orange were reinvestigated (Whiting, P. W., 1921) and the five fraternities involving linkage were summarized according to vials. Results are given in Table *E*. The difference between vials *a* and

TABLE *E*.

CROSS-OVERS OF A LETHAL LINKED WITH ORANGE ARRANGED ACCORDING TO AGE OF MOTHER.

	Vial.				
	a	*b*	*c*	*d–f*	Total.
Cross-overs	14/73	18/108	19/63	8/57	59/301
Total					
Per cent	19.2 ± 3.1	16.7 ± 2.4	30.2 ± 3.9	14.0 ± 3.1	

b is of no significance. There is an increase in cross-overs from *b* to *c*, 13.5 ± 4.6 per cent., which is probably significant and a decrease after *c*, 16.1 ± 5.0 per cent. which is also probably significant. If this rise and fall of crossing-over according to age is proved when further linked factors are found, it may be the most reasonable explanation of change in ratio of deficient.

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EXPLANATION OF PLATE I.

All figures have been drawn with camera lucida. Drawings have been reduced two thirds. Final magnifications are × 32 for Figs. *A–H*, × 15 for Figs. *I–M*.

FIGS. *A*, *B* AND *C*. Ventral view of head, ventral and posterior views of tip of abdomen of deficient male (Freak 60).

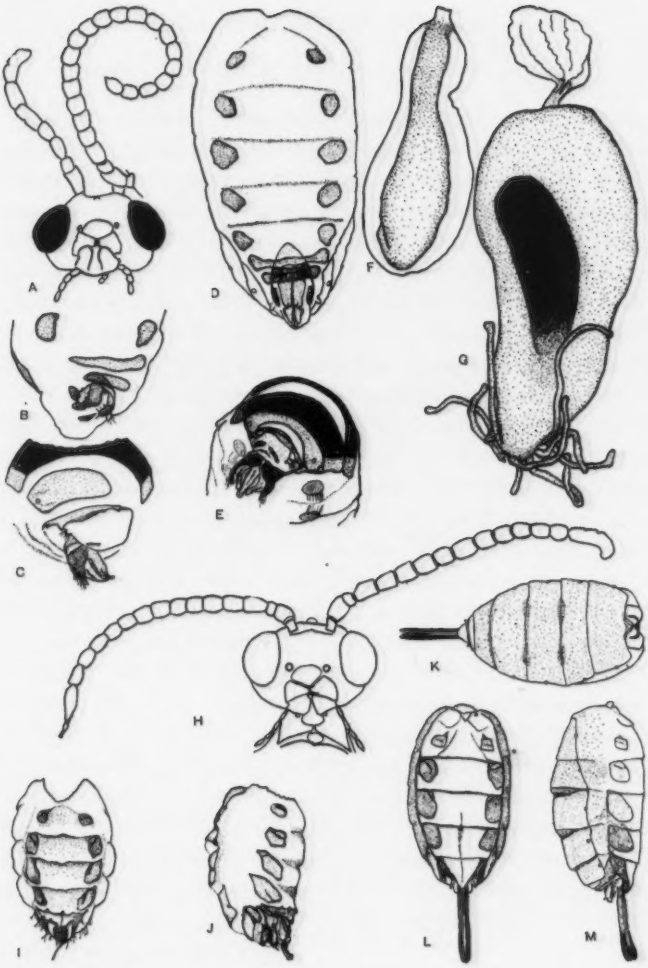
FIGS. *D* AND *E*. Ventral and posterior views of abdomen of normal male.

FIGS. *F* AND *G*. Abdominal portion of digestive tracts of deficient female (Freak 21) and deficient male (Freak 20).

FIG. *H*. Anterior view of head of deficient male (from 1171.3c).

FIGS. *I* AND *J*. Ventral and right views of abdomen of deficient female (Freak 6).

FIGS. *K*, *L* AND *M*. Dorsal, ventral and right views of abdomen of normal female.



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GAMETIC MEIOSIS IN *MONOCYSTIS.*

GARY N. CALKINS AND RACHEL C. BOWLING.

The monocystids of the earthworm, probably the commonest and most frequently found type of gregarine, have never been completely monographed, although an excellent start in this direction was made by Hesse (1909). Cytological studies have been made by Wolters (1891), Cuénot (1901), Shellack (1912), and Mulsow (1911), the latter finding typical gametic meiosis or chromosome reduction in a species which he regarded as new and to which he gave the name *Monocystis rostrata*. In this form which he named from the presence of an attenuated proboscis-like end of the cell, he found eight chromosomes of characteristic form and size. This number of chromosomes was found in the first nuclear spindle of the gamont after metamorphosis of the vegetative nucleus, and the same number was present in the successive spindles of the succeeding progametic nuclei and until the final division when the gametes are formed. In this ultimate division Mulsow found that whereas eight chromosomes are present in the nuclear plate of the mitotic figure at the metaphase only four chromosomes are present in the daughter plates of the anaphase stage and only four chromosomes are present in each of the two resulting gametes. Here then the haploid number of chromosomes is characteristic of only one set of cells, viz. the gametes, of the entire life cycle. Upon fusion of two gametes the diploid number is restored and this diploid number is characteristic of the entire vegetative cycle including all of the progametic nuclei. The actual reduction in number of chromosomes according to this account agrees in point of time with the corresponding meiotic phase in the great majority of the metazoa, a type which Wilson describes as gametic meiosis.

A very different time of reduction in chromosome number was described by Dobell in connection with gamete formation, fertilization, and metagamic divisions of the coccidian *Aggregata eberthi*, and by Jameson in connection with the gregarine *Diplo-*

cystis schneideri (Dobell and Jameson, 1915). This joint paper was followed later by individual papers on each of the two types (Dobell, 1925; Jameson, 1920). In both of these types the authors found that the progametic divisions are all alike including the last or gamete-forming division, in having nuclei with the same number of chromosomes, and that this number is the same in all of the vegetative stages. The zygotes, alone of all cells of the vegetative cycle, have the double or diploid number of chromosomes. Meiosis or reduction in number of chromosomes takes place with the first mitotic division of the zygote, a type known as zygotic meiosis. The individuals which one ordinarily encounters of these two species are therefore haploid as regards chromosome number.

From these findings Dobell and Jameson make the generalization that zygotic meiosis is probably universal in Gregarinida and Coccidia and they interpret Mulsow's account of reduction in *Monocystis rostrata* as evidence of confusion by him of two species of *Monocystis*, one with eight the other with four chromosomes. It seems a little premature to say the least to sweep away contradictory evidence in this fashion when a re-examination of *Monocystis* in the light of Dobell and Jameson's work would clear up the matter. The cytological results of such a re-examination are presented herewith.

There are many species of *Monocystis* in earthworms some of which are apparently limited to certain species of worms. We have restricted our investigation to the parasites of the seminal vesicles of one species—*Lumbricus terrestris*. Here several species of monocystid gregarines are found, some almost invariably present, others less frequently. We have frequently encountered the large form with a proboscis-like end which undoubtedly corresponds with Mulsow's *Monocystis rostrata* (450 μ long by 220 μ broad). We have not seen the long thread-like form which Hesse names *Nematocystis magna* (*Monocystis magna* Schmidt) and is said to be from 4.5 mm. to 5 mm. long and only 50 μ in diameter. We have not seen *Monocystis striata* Hesse with its longitudinal grooves and striations which are described as frequently prolonged posteriorly to form a tuft of filaments as in the genus *Zygocystis*. We often find *Monocystis agilis* which can

be easily identified and we often find another species which might be regarded as one of the varieties of *Monocystis lumbrici* which are not precisely described in the literature.

Cuénot (1901) Brasil (1905) and Hesse (1909) all remark on the difficulty of tracing a pair of pseudo-conjugants of *Monocystis* back to the individual agamonts which form the subjects of the specific descriptions. Where only one type of gregarine is present in an organ the assumption is safe that the cysts found in the organ come from that one type. When, however, there may be as many as five different species present at the same time, as is the case in the seminal vesicles of *Lumbricus terrestris*, then the problem is real. There is indeed a real danger of confusing sporocysts from different species and of getting a false interpretation of the life history of any one. On the other hand, if the species of *Monocystis* were based upon the size and structure of the sporocysts and sporoblasts instead of upon the agamont stages the difficulties of identification attendant upon multiple infection would be materially lessened.

While an ideal condition would be the indisputable association of a given agamont with its definitive sporocyst in these monocystids, for our purpose such an ideal is unnecessary. In all gregarines two individuals upon reaching maturity become associated in pairs (pseudo-conjugation) and a capsule, called the sporocyst capsule or simply sporocyst, is formed about them (Fig. 1). These gamonts then proceed to form gametes by repeated divisions of the originally single nucleus in each, the gametes ultimately appearing as a zone of small nucleated cells budded out from the periphery of the gamonts. The gametes from one parent cell then meet and fuse with the gametes from the associated cell. The zygotes thus formed by fusion two by two are practically equal in number to the total number of gametes formed by either one of the associated parent cells. Each zygote forms its individual capsule termed the sporoblast capsule within which the zygote nucleus, an amphinucleus, divides a certain number of times, usually three. The eight resulting nuclei become the nuclei of the definitive germs termed sporozoites.

It is no more difficult to recognize different specimens of the

same type of cysts and sporoblasts than it is to recognize different individuals of the same species of *Monocystis*. The structure of the agamont protoplasm, the nature of the sporocyst capsule, the

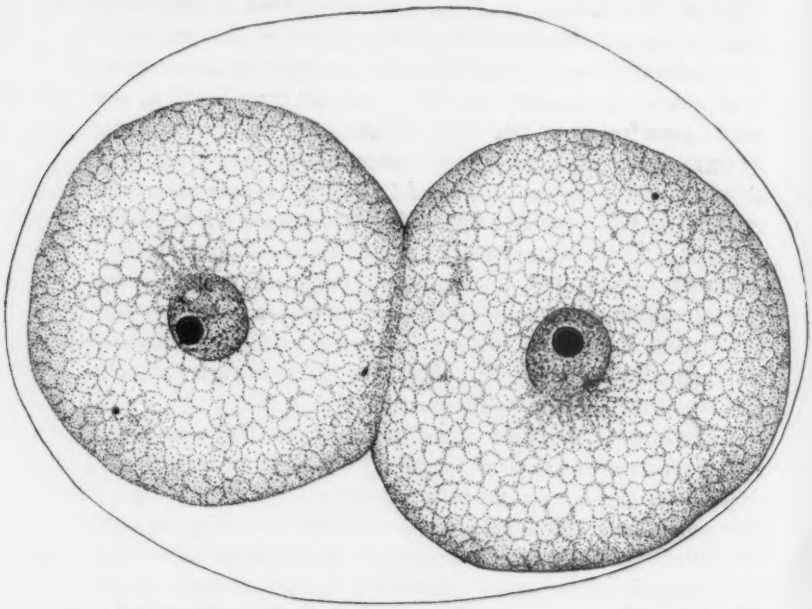

FIG. 1. *Monocystis* species. Two gamonts in pseudo-conjugation in sporocyst capsule. Nuclei beginning metamorphosis. Minute spindles in substance of centrosphere. Smear preparation. Camera lucida. × 900.

appearance of the nuclei in size and character together with the number of chromosomes, are factors involved in the recognition of a cyst of a certain species. We have been no more fortunate than our predecessors in tracing the indisputable connection between a specific agamont and the sporozoites which it ultimately forms and for this reason we beg the question of the particular species of *Monocystis* with which we are dealing. We are confident however that we have followed the history of one single species.

We have chosen for particular study the cysts containing the largest sporoblasts and the largest gametes because their nuclei

give the clearest pictures of the nuclear contents. The total number of gametes formed by both gamonts in this species is approximately 130 and the number of sporoblasts is approximately 65. The protoplasm is uniformly alveolar, clear, and with alveoli of medium size. The nucleus of the gamont is spherical and contains a single spherical endosome.

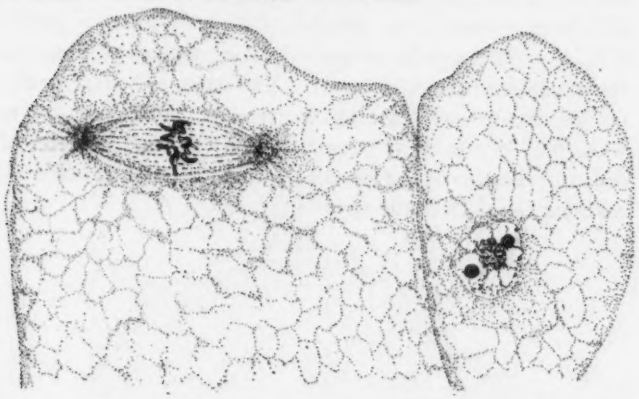

FIG. 2. First pregametic spindle with 10 chromosomes in one gamont; portion of disintegrating nucleus in the other. Section. Camera lucida. × 2650.

The material was fixed in hot Schaudinn's fluid consisting of a saturated solution of bichloride of mercury in absolute alcohol without and with 5 per cent. of glacial acetic acid. Seminal vesicles were removed entire from richly infected earthworms and immersed after puncturing, in the killing fluid where they were left for from two to four hours. They were then treated for twenty-four hours with iodized alcohol, embedded in paraffine and cut in sections from three to four microns thick. The sections were stained with iron hæmatoxylin without or with a counter stain. Other stains were tried but the best results were obtained with iron hæmatoxylin.

The metamorphosis of the gamont nucleus begins with the condensation of the chromatin and formation of the chromosomes. At this stage the latter are feebly staining and even in the metaphase of the first division they do not stain with the same intensity as in the later progamous divisions or as they do in the

developing zygote. An astrosphere is condensed from the cytoplasmic reticulum at one pole of the nucleus and the nuclear membrane disappears at this region. We did not observe a stage in which a single centriole could be found in the astrosphere, the earliest stage seen being a minute spindle which appeared to be formed in the substance of the astrosphere in the region which had been occupied by the now dissolved nuclear membrane (Fig. 1). This spindle enlarges until it occupies fully one half of the diameter of the cell (Fig. 2). The short, rod-shape or slightly curved chromosomes are ten in number. The daughter nuclei when fully divided may be separated by the full diameter of the cell, and remains of the spindle substances trail out behind them marking the path of the spindle (Fig. 3).

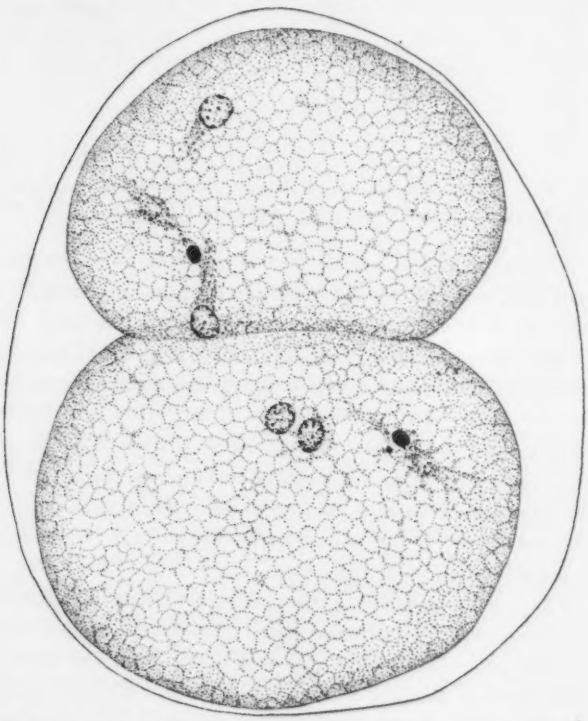

FIG. 3. Two gamonts after first pregametic nuclear division. Smear. Camera lucida. × 900.

The subsequent spindle figures are characterized by the increasingly intense staining capacity of the chromosomes while the size of the nuclei decreases. There is a tendency for the

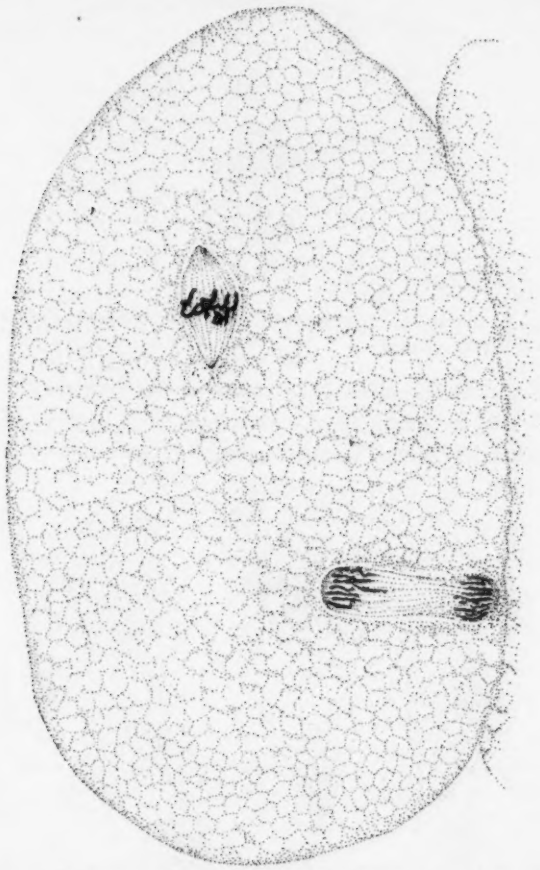

FIG. 4. Third pregametic division of nuclei with 10 chromosomes. Section. Camera lucida. × 1750.

daughter nuclei after each division to approach the periphery and the final progamous nuclear spindle lies in a plane tangential to the surface (Figs. 4 and 5). In some cases the spindle occupies a bud which stands out from the surface in characteristic fashion

(Fig. 7). Such tangential nuclear figures are typical of the final progamous division, and their products become the gamete nuclei.

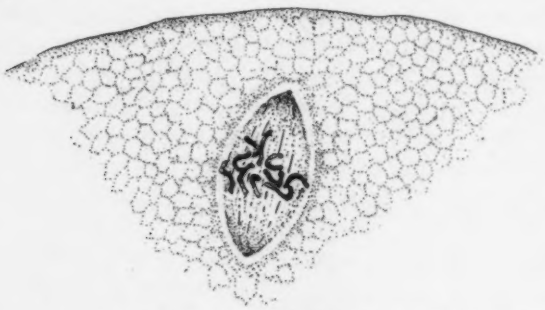

FIG. 5. Detail of a spindle during the third pregametic division. Section. Camera lucida. × 2650.

In the tangential nuclei as in the earlier pregametic nuclei we have seen no evidence of a prophase skein stage. Metaphase stages of division are frequently found (Fig. 6) and early and late

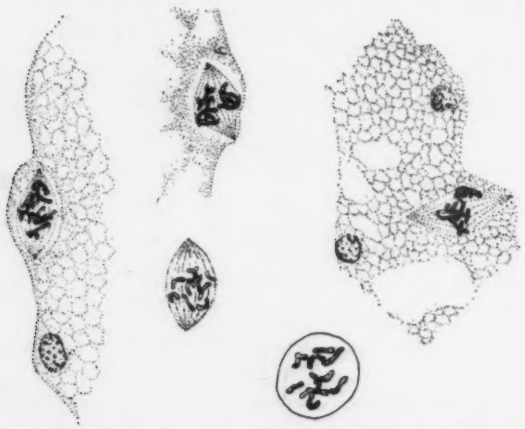

FIG. 6. Group of spindles in the metaphase stage of the last pregametic division, one showing polar view of a nuclear plate. From different individuals; all with 10 chromosomes. Sections. Camera lucida. × 1750.

anaphase stages. In these the number of chromosomes is always ten (Figs. 7 and 8). In the metaphase stage of the tangential

spindles there are likewise ten chromosomes, but these ten do not divide longitudinally. They are separated into two groups of five chromosomes each, easily distinguishable in the anaphase

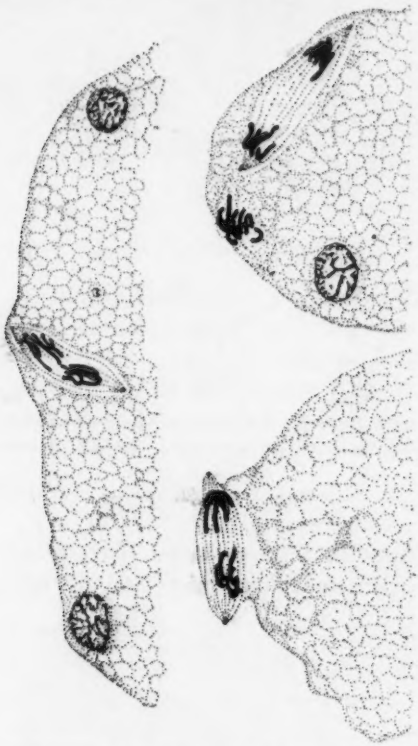

FIG. 7. Group of spindles in the anaphase stage of the last pregametic division; from different individuals. 5 chromosomes in the daughter plates. Sections. Camera lucida. × 1750.

groups (Figs. 7 and 8). Reduction in number of chromosomes from 10 to 5 therefore takes place with the last progamous division.

The chromosomes are not sufficiently different in shape and size to furnish any evidence of coupling. Two daughter groups are represented in Fig. 8; some are U-shape others are usually somewhat sigmoid in character, but the differences are not so

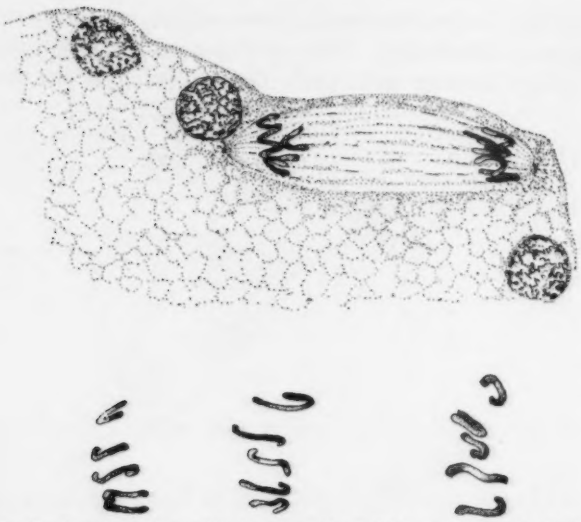

FIG. 8. Anaphase stage of last pregametic division and 3 groups of chromosomes from other spindles in the same stage. Sections. Camera lucida. × 2650.

marked as in the case of *Aggregata* or as in Mulsow's *Monocystis rostrata*.

The gametes are relatively large, spherical to ovoidal in shape and without constant dimorphism although minor variations in

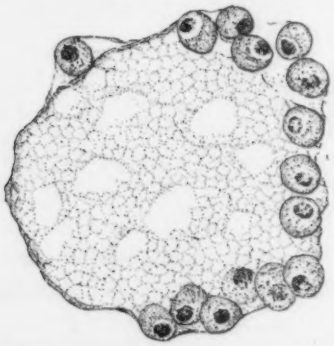

FIG. 9. Characteristic grouping of gametes around periphery of gamont. Section. Camera lucida. × 600.

size may be observed even in gametes from the same gamont (Fig. 9). The nuclei are usually near the anterior end of the cell

(Fig. 10) and when two gametes come together, fusion begins at this nucleated end. The nuclei fuse and the zygote secretes a delicate plastic membrane which is transformed later into the rigid sporoblast capsule (Figs. 11, 12 and 13). At this early

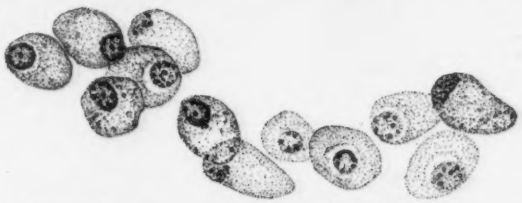

FIG. 10. Group of gametes. Smear. Camera lucida. × 900.

period of the zygote the form is quite variable owing to the plasticity of the membrane (Fig. 15). The amphinucleus swells and becomes more conspicuous by reason of the more intensely staining chromatin. The chromomeres become arranged in a loose spireme which appears to be continuous (Fig. 14). The

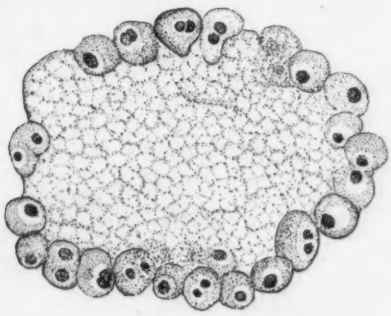

FIG. 11. Characteristic grouping of gametes and zygotes about gametocyte. Section. Camera lucida. × 600.

threads are double and break up into 10 chromosomes. A spindle is formed with definite poles and fibrils but the latter are not as sharply defined as in the progamous divisions (Fig. 15). In the anaphase stage 10 chromosomes are present in each daughter plate (Fig. 14).

After this first metagamic division, which is not accompanied by cell division, we were unable to make out any definite spindle

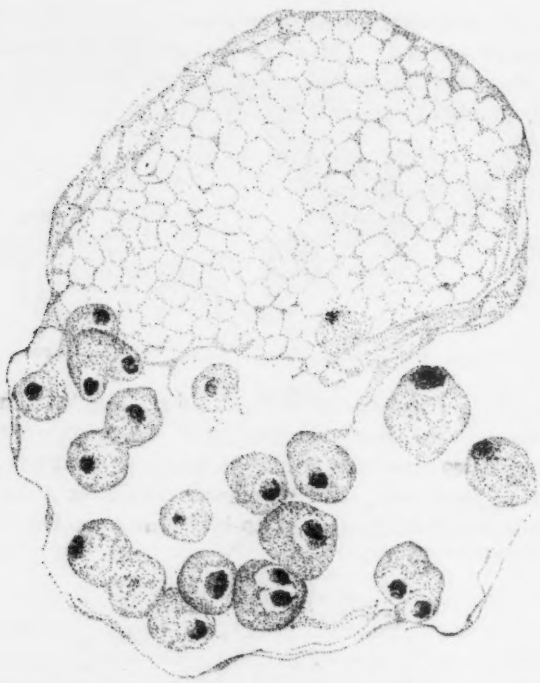

FIG. 12. Group of gametes undergoing fusion to form zygotes. Section. Camera lucida. × 900.

FIG. 13. Group of zygotes with stages in fusion of the nuclei. Section. Camera lucida. × 1750.

formation. The two daughter nuclei soon divide again, an inference formed by reason of the comparatively rare occurrence of the binucleated sporoblast. The two nuclei give rise to four

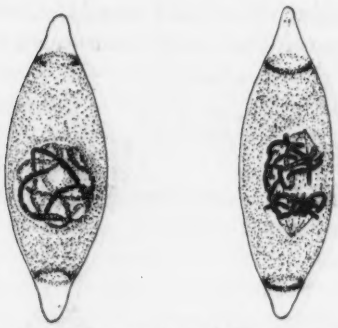

FIG. 14. Young sporoblasts, one with nucleus in spireme stage, the other with nucleus in anaphase stage of division with 10 chromosomes in each daughter plate. Section. Camera lucida. × 2300.

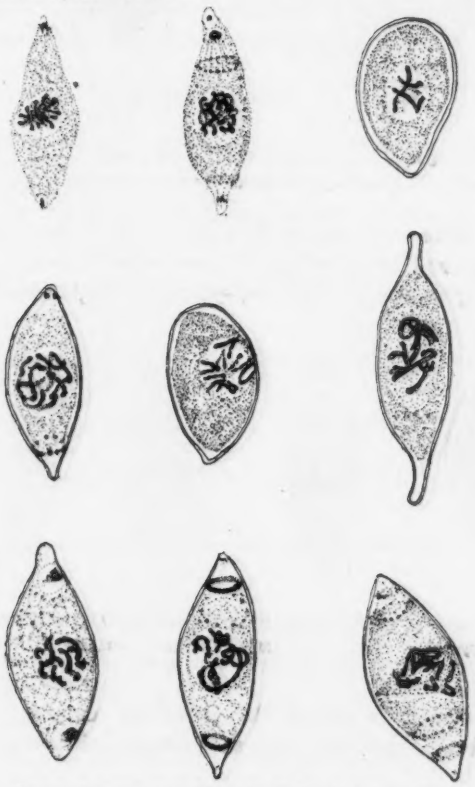

FIG. 15. Group of young sporoblasts in different stages. Sections. Camera lucida. × 1750.

without division of the cell and such sporoblasts with four nuclei are much more common than are the binucleated ones (Fig. 16).

FIG. 16. Group of developing sporoblasts, 2 with 2 nuclei, 2 with 4 nuclei and one with 8 nuclei—no cytoplasmic division. Section. Camera lucida. × 1750.

A third division gives rise to the eight nuclei of the sporozoites and at this stage the cytoplasm divides into eight elongated cells each bearing a nucleus; each is a sporozoite (Figs. 16 and 17).

FIG. 17. Group of late sporoblasts from one sporocyst, each with 8 sporozoites In one the sporoblast suture is clearly indicated. Section. Camera lucida. × 1750.

During this later period the sporoblast capsule becomes definitely formed and rigid; near each pole a distinct ring of more

intensely staining substance is conspicuous. The origin and significance of these rings will be considered in a later paper. In old sporoblasts a definite suture or groove runs down the sporoblast capsule on both sides from pole to pole, indicating the presence of two valves and a prospective opening by dehiscence (Fig. 17).

The chromosome history of this gregarine thus does not bear out the generalization made by Dobell and Jameson that zygotic meiosis is probably characteristic of all gregarines. It is more probable that both zygotic and gametic meiosis occur in this group, the former probably in forms like *Echinomera hispida* (Shellack, 1907) which has 5 chromosomes, in *Nina gracilis* (Léger and Duboscq, 1909) which has 5 chromosomes, and in *Monocystis ovata* (Shellack, 1912) which has 3 chromosomes, and in other forms with similar odd numbers of chromosomes.

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PRELIMINARY NOTE ON A NEW PROTOZOAN PARASITE OF EARTHWORMS OF THE GENUS *EUTYPHŒUS*.

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In the course of a recent study of the earthworms of Burma a large protozoan parasite of unusual form was frequently encountered. The writer can discover no reference to any parasitic animal of similar form in the literature. The following description will therefore be of interest to zoölogists, although it should be understood that the present paper attempts nothing more than the presentation in a preliminary way of data gathered during a study of the host animal.

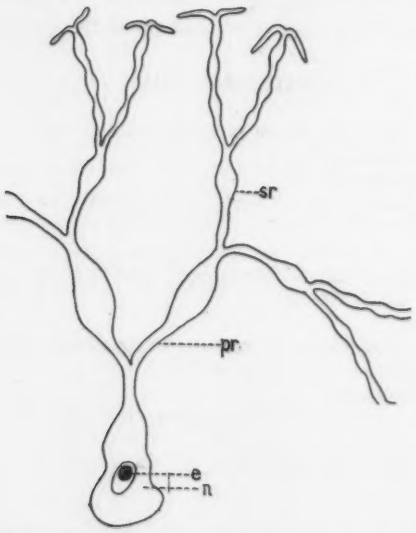

FIG. 1. Part of a trophozoite. (Camera lucida.) *s.r.*, secondary ramus; *p.r.*, primary ramus; *n.*, nuclear membrane; *e.*, endosome.

The parasites may be as long as 4 mm. To the unaided eye

the animal presents a smooth creamy-white appearance, or very rarely, a light brownish tinge. The latter color, however, is probably due to a staining action on the parasite of some substance from the host. The large unattached end of the animal is bluntly rounded. At a greater or less distance from this end the body branches into two rami each of which is narrower than the main trunk (Fig. 1). Each of the two primary rami branch again to form two smaller secondary rami. This branching continues until eight or sixteen small ramuli are produced. The region at which ramification begins is not constant in position.

FIG. 2. Sucker-like organs of attachment. (Camera lucida.) × ca. 475.

In one specimen which is 1.4 mm. long the first branching occurs at a distance of about .9 mm. from the blunt end, the second 1.2 mm., and the third 1.32 mm. In other specimens the trunk portion is often much shorter proportionately, rarely even shorter than the primary rami. The ramuli at the attached end are the shortest, the rami usually increasing in length towards the free end of the parasite. The dichotomy is regular in all specimens examined. The ramuli bear groups of irregularly-ovoid, sucker-like objects by means of which the parasite is attached to the host (Fig. 2).

Longitudinal and transverse striations are visible in the ectoplasm with the high powers of the microscope. The transparent fluid endoplasm of the trunk and primary rami is densely packed with ovoid paraglycogen granules. These granules are widely separated in the secondary rami and only very sparsely distributed in the ultimate, transparent branches.

The nucleus (Fig. 3) is a large, ovoid body sometimes visible to the unaided eye in cleared specimens as a minute spot. In large specimens it is .64–.86 mm. in length. The contents of the nucleus are perfectly transparent except for a single, eccentrically located, spherical body of light bluish appearance.

This endosome has either one large, or several smaller, transparent, vacuole-like areas. The nuclei of dead parasites are always found in the unbranched portion.

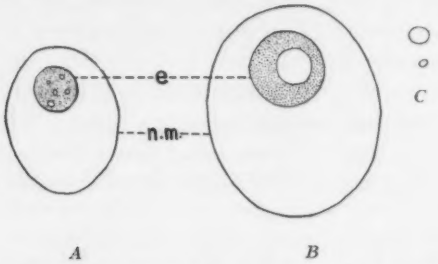

FIG. 3. *A*. Nucleus from trophozoite 1.3 mm. in length. (Camera lucida.) × ca. 335. *e.*, endosome; *n.m.*, nuclear membrane. *B*. Nucleus from larger trophozoite. (Camera lucida.) × ca. 350. *C*. Paraglycogen granules. (Camera lucida.) × ca. 350.

Live attached parasites have not been studied but in living detached forms two series of movements are noticeable. The first series may be described as waves of peristaltic contraction passing along the trunk and rami of the first order. These peristaltic contractions produce a back and forth churning of the endoplasmic contents in the trunk, from the trunk into the primary rami, and back and forth in the rami. By these contractions the nucleus is squeezed to and fro from one end of the trunk to the other. In two of the living specimens examined the nucleus remained in the unbranched portion of the parasite during the whole period of observation. In the other animal the nucleus frequently passed into one or the other of the primary rami. In the primary ramus the nucleus was either rolled back and forth several times or at once passed back into the trunk. The nucleus moved with equal ease and frequency into either one of the primary rami but never passed into the secondary rami which were narrower than the nucleus. It should be noted that in this third animal the ramification began unusually near the blunt end so that the trunk portion was less than one third of the total length of the parasite.

The second series of movements may be called rotation movements and result in a spiral twisting of the primary rami and their branches back and forth on their own axes.

The parasites occur in the cœlomic cavities of one genus of Burmese earthworms, *Eutyphœus* and most commonly in *E. foveatus*. They are found in smaller numbers and more rarely in *E. spinulosus*, *rarus*, and *peguanus*. One hundred per cent. of the *E. foveatus* examined during a period extending over four years have been infected. In the majority of worms of the last named species, large numbers of parasites are found, masses containing 20–40 individuals being not uncommon. The largest groups of parasites occur in segment three, the majority of which are attached to the large nephridial masses of this segment. Other locations are: under the nerve cord in segment four, and on the septa or parietes of segments four to twenty. Those found posterior to segment nine are almost always less than one millimeter in length.

AIKINETOCYSTIS SINGULARIS, n. gen., n. sp.

Diagnosis.

Uninucleated protozoa parasitic in the cœlomic cavity of certain earthworms of Burma; body cylindrical or columnar with a characteristic, regularly dichotomous branching at the attached end; fixation to the host by means of sucker-like bodies borne on the ultimate branches; nucleus with a single eccentrically placed endosome.

Additional Observations.

Pairs of animals are frequently found attached to each other near the rounded free ends. Among the larger sized forms such pairs are nearly as common as single animals, but do not occur among the smaller parasites. Groups of three to eight animals similarly adherent to each other are also found. Attempts to pull apart the attached animals have always resulted in breaking one or both of the animals near the place of attachment. However no evidence of organic connection between members of a pair or group has been found.

Monocystid-like spores of typical pseudo-navicella form in masses visible to the naked eye are sometimes present in segments three to five. In other specimens in which such masses are not visible similar spores can almost always be found by scraping the body wall of the segments just mentioned. The spores are of

two sizes: the larger .02–.023 mm. long, the smaller .007–.008 mm. long. The contents of the spores are coarsely granular. A single transparent vesicular body is present, centrally placed in the small spores, eccentrically placed in the large spores. As no other parasites with a cyst- or spore-forming habit are found in segments three to five and as the spores are practically always present with the trophozoites, it may be inferred, at least tentatively, that they are produced by the trophozoites with which they are always associated.

Numerous specimens of the host have been dissected in every month in which the worm can be obtained but only three cysts from as many worms have been found in proximity to the trophozoites. The cyst in each case was ovoid in shape and about .62 mm. long. A tough membrane enclosed a fluid in which floated two hemispherical bodies in contact with each other on a flattened surface, nearly filling the cavity of the cyst. After rupturing the membrane the gamonts were easily separated from each other. The single hemisphere retained its shape and opacity in clearing fluid. Rupturing the hemisphere by pressure on the cover-glass released granules similar in appearance to those found in the endoplasm of the trophozoite, but no nucleus or spores could be seen. Spore-containing sporocysts have not been found in the anterior segments but cysts about .75 mm. long and .6 mm. wide, densely packed with spores similar in size and appearance to those occurring in the anterior segments are sometimes present in the whitish masses filling the cœlomic cavities of the anal segments.

Worms of the genus *Eutyphœus* occurring in Rangoon suddenly disappear at the end of the rainy season and are not to be found again until after the beginning of the next rainy season. The failure to find the sporocysts in spite of the constant presence of the spores may be due to a coincidence of the sporocyst formation with this winter hibernation period of the host.

Summary.

A new protozoan parasite of the cœlomic cavities of certain tropical earthworms, with a remarkable branched structure of the trophozoite is described. Mention is made of associated spores and sporocysts possibly belonging to the trophozoites.

A CYTOLOGICAL STUDY OF SECRETORY PHENOMENA IN THE SILK GLAND OF *HYPHANTRIA CUNEA*.

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INTRODUCTION.

The general anatomy and histology of the silk glands of lepidopterous larvæ have been carefully studied and described by numerous investigators beginning with the work of Helm ('76), but little purely cytological study of the cells with an aim toward giving an account of the mechanism of secretion was reported before the investigations of Maziarski ('11) and later of Nakahara ('17). Since that time, few papers have appeared based upon studies of the silk gland, with the exception of an account by Yamanouchi ('22) describing a morphological study of silk secretion.

In previous investigations of silk secretion, studies have been made only upon glands of caterpillars which spin a cocoon at a certain period in their life history. Such study is of value in observing glandular function and development during the progressive stages preparatory to and at the time of activity. In order to best study the actual process of secretion, the optimum material would naturally be found in a form which secretes actively over a comparatively long period of time.

The fall webworm, *Hyphantria cunea*, is ideally adapted for the study of active secretion, for the caterpillars live together in colonies, secreting the web continually throughout larval life in the process of securing food. This form, therefore, was selected for the present investigation and by the application of cytological methods, an attempt has been made to determine the cellular organization which is concerned with secretory phenomena in the silk gland.

Work upon this problem was for the most part carried on during 1925 and 1926, at Washington University, St. Louis,

under the direction and supervision of Prof. Caswell Grave. It is with great pleasure that I take this opportunity to express to Dr. Grave my sincere appreciation for his advice, criticisms, and valuable suggestions.

Material.

The spinning-glands of the larval *Hyphantria cunea*, like those of all lepidopteran larvæ, consist of two long tubes, easily distinguishable by their glassy appearance, situated on either side of the nerve cord and below the digestive tract. Each tube is a cylindrical, folded body, averaging between 6 and 8 mm. in length, or slightly less than the entire length of the caterpillar which averages about 1 cm. In total mounts of a spinning-gland, three arbitrary divisions may be distinguished, characterized by differences in appearance, and, in section, by structure (Text-Fig. *D*); the posterior, or secreting portion lying between the eighth and eleventh segments and terminating blindly; the middle portion or reservoir, bending posteriorly to the ninth or tenth segment, then folding anteriorly to the fifth segment; the anterior, or conducting portion extending straight forward to the head where the two tubes converge and unite to open at the apex of a median cylindrical organ, the spinneret.

Each tube consists of a single layer of epithelial cells (Imms, '25, p. 407), varying in shape and size according to position in the tubule. The cells are arranged in two longitudinal rows around a central lumen. The nucleus of each cell is characteristically branched and very irregular. Externally, the gland is covered by a thin membrane, the tunica propria, and internally, the lumen is lined by the thicker tunica intima.

The cells of the posterior region are relatively large and irregular in form and contain a greatly branched nucleus. The inner cell wall is irregular in surface contour, due to numerous drops of secretion in passage between the cell and lumen. The tunica intima is not readily distinguishable (Text-Fig. *A*, *B*). The cells composing the middle region are greatly flattened with a consequent elongation of the nucleus, and possess a clear hyalin, inner border. The cavity of the lumen has increased in diameter and is filled by secreted material (Text-Fig. *C*, *D*).

The cells of the anterior conducting portion are reduced in size, being compressed but less elongated than cells of the reservoir. The nuclei tend to be rounder and less branched, and the intima can be definitely distinguished (Text-Fig. *E*, *F*).

The tracheæ that supply the posterior and middle regions penetrate through the tunica propria into the substance of the cell. It is significant that no intracellular tracheæ are present in the anterior conducting portion of the silk glands.

COLOR REACTIONS OF SILK GLAND CELLS WITH VARIOUS STAINS AND FIXATIVES.

Fixative and Stain.	Chromatin.	Nuclear Bodies.	Nucleoloid Bodies.	Mitochondria.
Benda:				
Delafield's Hæm., Acid Fuchsin..	Blue	Black	Black	
Ehrlich's Hæm., Acid Fuchsin....	Blue	Blue	Blue	
Ehrlich-Biondi.....	Pink	Pink	Orange	
Iron Hæm., Erythrosin.....	Black	Black	Black	Black
Bouin:				
Delafield's Hæm., Acid Fuchsin..	Blue	Blue	Blue	
Ehrlich's Hæm., Acid Fuchsin....	Blue	Blue	Black	
Iron Hæm., Acid Fuchsin.....	Pink	Black	Black	
Champy:				
Altmann, Acid Fuchsin, Methyl gr.....	Green	Red	Red	Red
Delafield's Hæm., Acid Fuchsin..	Blue	Black	Black	
Ehrlich's Hæm., Acid Fuchsin....	Blue	Blue	Blue	
Ehrlich-Biondi.....	Pink	Red	Orange	
Iron Hæm., Acid Fuchsin.....	Black	Black	Black	Black
Gatenby:				
Delafield's Hæm., Acid Fuchsin..	Black	Black	Black	
Iron Hæm., Acid Fuchsin.....	Black	Black	Black	Black
Mann-Kopsch:				
Unstained.....	Yellow	Yellow	Yellow	
Altmann, Acid Fuchsin, Methyl gr.....	Reddish	Reddish	Brown	
Regaud:				
Altmann, Acid Fuchsin, Methyl gr.....	Green	Red	Green	Red
Iron Hæm., Erythrosin.....	Pink	Black		Black

Larval *H. cunea* furnished the entire supply of material with the exception of a few glands from *Apatella americana* and *Diacrisia virginica* which were preserved in Bouin and DaFano preparations for comparison. The age of the webworms could not be determined as they had been actively secreting for some time previous to collection as evidenced by the size of the webs. An abundant supply of material was secured from bayberry

COLOR REACTIONS OF SILK GLAND CELLS WITH VARIOUS STAINS AND FIXATIVES.

Fixative and Stain.	Cytopl. Bodies.	Secretion.		Intima.	Mucoid Sub.	Cytopl.
		Outer.	Inner.			
Benda:						
Delafield's Hæm., Acid Fuchsin	Pink	Light brown	Olive	Pink	Pink	Pink
Ehrlich's Hæm., Acid Fuchsin			Olive	Olive		Pink
Ehrlich-Biondi	Pink	Pink	Blue-gray			Green
Iron Hæm., Erythrosin	Black		Black	Pink	Pink	Pink
Bouin:						
Delafield's Hæm., Acid Fuchsin	Pink	Olive	Pink			Pink
Ehrlich's Hæm., Acid Fuchsin	Pink	Pink	Dark pink	Pink	Pink	Pink
Iron Hæm., Acid Fuchsin	Black	Black	Pink	Black	Pink	Pink
Champy:						
Altmann, A. Fuch., Methyl green	Red	Dark red	Light red	Green	Green	Green
Delafield's Hæm., Acid Fuchsin		Pink	Brown	Pink	Pink	Pink
Ehrlich's Hæm., Acid Fuchsin	Bluish	Pink	Olive	Pink	Pink	Pink
Ehrlich-Biondi	Pink-green	Red-orange	Green-gray	Green	Pink	Green
Iron Hæm., Acid Fuchsin	Black		Black	Pink	Pink	Pink
Gatenby:						
Delafield's Hæm., Acid Fuchsin		Dark brown	Light brown	Pink	Pink	Pink
Iron Hæm., Acid Fuchsin	Black		Black	Pink	Pink	Pink
Mann-Kopsch:						
Unstained	Black		Olive	Brown	Yellow	Yellow
Altmann, A. Fuch., Methyl green	Olive	Olive	Red	Green		Green
Regaud:						
Altmann, A. Fuch., Methyl green	Red	Green	Red-green	Red		Green
Iron Hæm., Erythrosin	Black	Black	Black	Black		Pink

bushes on Nonamessett Island, Mass., between August 13 and 16, and brought into the laboratory at Woods Hole where the animals were kept supplied with food and moisture until killed.

Methods.

The caterpillars were killed by piercing the head with a pin after which they were immediately fastened in a paraffined dish containing Locke's solution, opened by a dorsal incision, and the glands removed directly with glass needles and placed in fixing fluids. Several fixing solutions were used, namely; Bouin's, Zenker's, Gilson's, for general structure, Benda's, Gatenby's modified "Flemmings" for cell inclusions, Champy's method for preserving and differentiating Golgi bodies and mitochondria, and Regaud for mitochondria.

Transverse and longitudinal sections, 4 micra in thickness, were made of the glands and stained by various methods. The results of different combinations of fixatives and stains may be seen from the chart.

Observations.

1. *The Nucleus.*

(*a*) *Historical.*—The nucleus of larval spinning-gland cells has been found to be characteristically large and greatly branched; the branches extending toward all sides of the cell. This peculiarity of shape early attracted investigators and led them to study the nucleus and its contents in an effort to find some possible relation between its unusual form and the active secretion of silk; but it was not until Korschelt in 1896 published the results of his observations upon living and fixed material that a detailed account of the nuclear content of spinning-gland cells appeared in the literature. From the results obtained through use of a modification of the Ehrlich-Biondi stain, Korschelt concluded that the larger bodies or macrosomes found in the nucleus correspond to the chromatin, and the smaller bodies, or microsomes to nucleoli. Meves ('97) used other staining reagents and came to opposite conclusions. He identified the macrosomes with the nucleoli and the microsomes with chromatin. More recent investigations have seemed to support

the conclusions of Meves (Marshall and Vorhies, '06; Vorhies, '08; Maziarski, '11; Nakahara, '17).

(*b*) *Observations.*—Within the nuclear membrane, which can usually be distinguished with little difficulty in the spinning-gland cells of *H. cunea*, bodies of two sizes are always present (except in the anterior conducting region) and with great regularity and frequency a larger, third body is also found (Fig. 5). The smallest bodies are by far the most abundant and tend to fill the entire nucleus. With certain fixatives, Bouin, Zenker, a reticulum can be distinguished, upon which are arranged these smallest bodies. This is not the case, however, after fixation in Regaud, Champy, Benda, or Gatenby, for in the middle and posterior regions of the gland the bodies always appear to be free in the nucleus, forming a compact mass of small granules, and take a basic stain. (See chart.) These bodies have been considered as chromatin. In the anterior region of the gland, these bodies are consistently present although considerably more scattered.

The second group of bodies present in the nucleus exhibits considerable variability both of size and distribution. These are the macrosomes or nucleoli of previous workers, but in this paper, they will be referred to as "nuclear bodies." It is only rarely that these nuclear bodies are found in the nuclei of the conducting cells, and when they chance to be present, are considerably smaller than similar bodies in the posterior or middle regions. No general rule can be given for the shape or dimensions of these nuclear bodies as they tend to vary greatly. Their usual shape is spherical or ellipsoid, and this is typical of their form in the middle region, but toward the posterior end of the gland where there are evidences of active secretion indicated by droplets in the lumen and on the edge of the lumen, these bodies are often elongated and irregular in shape. In size, there is variation in length between 0.5 micron and 2 micra and occasionally a few are larger. Even within the same nucleus, such variation in size is present. The bodies have no special arrangement in the nucleus but tend to be dispersed throughout the area, very often approaching the nuclear membrane. They have even been seen penetrating the nuclear wall into the cyto-

plasm. Critical observation with high magnification definitely showed nuclear bodies partly within the nucleus and partly in the cytoplasm (Fig. 4). The passage of nuclear bodies into the cytoplasm is a fact that has been described with great care by Maziarski ('11), Nakahara ('17), and earlier workers, as of regular occurrence in the spinning-gland cell; by Saguchi ('20) as occurring in nuclear function of the pancreas cell; and by Ludford ('25) in tissue cultures of fibroblasts of the rats' kidney.

The staining reaction of these nuclear bodies is of interest because of the fact that although it is more or less variable, yet it tends to show greater affinity for acid stains. (See Chart.) It has been upon reactions to stains that all the conclusions in regard to the relation between nucleus and secretion have been made, since it has been regularly found that the nuclear bodies (nucleoli) and apparently related bodies in the cytoplasm exhibit the same staining reactions.

The third class of nuclear bodies found in *H. cunea* has not been described in the literature so far as it has been possible to determine. Because of the similarity between these bodies and the true nucleoli, they will be referred to throughout this paper as "nucleoloid bodies." Meves ('97) mentions the fact that nucleoli may contain several small vacuoles, but in the figures of nucleoli in which he shows this structure, they are no larger than numerous other bodies in the nucleus. Korschelt ('97) in replying to the criticism of Meves, attempts to explain the presence of vacuoles by the hypothesis that the nucleoli or microsomes are derived from the chromatic substance by some transformation evidenced, no doubt, by these vacuoles. Marshall and Vorhies ('06) definitely state that no plasmosome or special structure is formed in the nucleus during secretion in spinning-gland cells of *Platyphylax*, but mention the fact that sometimes the nucleoli may contain vacuoles but no further significance is attached to the observation; nor did Nakahara ('17) consider as important the presence of vacuoles in nucleoli. In the nuclei of *H. cunea*, vacuolated bodies are found to be sufficiently definite to attract especial attention. After carefully measuring a large number of such bodies in all parts of the silk gland, it was found that the average diameter is about 4 micra regardless

of position in the gland. Several measured 3 micra in diameter and a very few in the more actively secreting region are as large as 5 micra or 6 micra, while in the anterior region, these bodies average about 2 micra in diameter. This size relation is constant regardless of the fixation used.

Each nucleoloid body contains two or more vacuoles or clear spaces which either do not stain or, if so, only faintly. In a section of a nucleus, usually only one of these bodies is present, rarely two (Figs. 3, 5, 6), and the frequency with which they occur in serial transverse sections 4 micra in thickness leads to the conclusion that not more than two are usually present in a nucleus. This point has been very difficult to determine accurately on account of the uncertainty in deciding where one nucleus ends and the next begins. From longitudinal sections, the evidence was more definitely in support of this conclusion. These nucleoloid bodies are either centrally or just eccentrically placed, and are never found in close approximation to the nuclear wall. Their usual shape is spherical, thus distinguishing nucleoloid bodies from nuclear bodies which tend to vary in shape. From careful study, it has appeared that the presence of nucleoloid bodies is usually associated with an increase in the number of nuclear bodies (macrosomes) either in the same section or the next in series. In the immediate vicinity of the nucleoloid body, the nuclear bodies are smaller, increasing in size toward the periphery of the nucleus (Figs. 3, 5, 6). The periphery of the nucleoloid bodies is not always regular, for often protrusions apparently in the process of pinching off may be seen (Fig. 5).

That the vacuolated nucleolus may give rise to nuclear bodies is not without precedent in observations. Saguchi ('20) in the pancreas cell, describes a very similar process which gives rise to nucleolar corpuscles: p. 355: "As regards the genesis of the nucleolar corpuscles, I am fully convinced that they are derived from the main or side nucleoli." Montgomery ('99) concludes that the nucleolus may act as a nuclear organ either of excretion or storage and that when it has enlarged to a certain extent, it constricts pieces from itself.

The nucleoloid bodies usually react more specifically to basic

stains (see chart), but occasionally with Altmann's anilin acid fuchsin, methyl green after fixation by the Regaud method, bodies can be found staining deeply green at the periphery and pink in the center.

(*c*) *Critical.*—From observations upon silk gland cells of *H. cunea*, it would seem that the bodies which migrate from the nucleus into the cytoplasm are not nucleoli, but are rather products of nuclear activity. That this is placing too great a burden upon a single small organ of the nucleus may be argued, but it need not necessarily follow that all the products of secretion are derived from the nucleoloid bodies, but rather that the latter contribute bodies which enlarge in the nucleus, pass out into the cytoplasm where they undergo further modification and later become discharged into the lumen. In order to verify the concluding part of this statement, a general survey of the cytoplasm and its contents is necessary.

2. *The Cytoplasm.*

(*a*) *Cytoplasm.*—The cytoplasmic structure of the silk gland cell as of any cell, is difficult to describe in concrete terms for it is more or less affected by fixation and the exact or true nature is hard to determine. After fixation with the Champy, Gatenby, Benda, or Regaud methods, the cytoplasm is homogeneous and granular, but after Bouin and Mann-Kopsch fixation, a definitely striated appearance may be distinguished, the striæ extending from the lumen toward the periphery. No evidence of fibrils have been found in any case. Yamanouchi ('22) definitely describes fibrillæ in the protoplasm between which are situated granules, especially toward the lumen. These, he explains, are cross sections of fibrillæ and that the entire structure is composed of minute fibrillæ arranged in rows. The usual staining reaction is acidophilic. (See chart.)

(*b*) *Granules.*—In the posterior region of the gland, throughout the cytoplasm, there appear spherical bodies either enclosed in vacuoles or apparently free in the protoplasm (Fig. 1). Maziarski ('11), Nakahara ('17), and others, noted this condition and from the fact that the bodies are often found in close proximity to the nucleus and exhibit the same staining reactions

as the nuclear bodies drew the conclusion that the cytoplasmic granules are derived from the nuclear bodies (nucleoli) by migration of the latter. Tanaka ('11) believes the fibroin, or silk core, to be a product of the gland cell substance, visible only when the secretion has accumulated to give rise to fine fibroin droplets which unite to form larger droplets. Yamanouchi ('22) likewise believes that secretion is produced in the cell.

Whether or not these cytoplasmic bodies are produced in the cell or in the nucleus, it seems to be generally concluded that they are directly connected with the products of secretion.

From observation of cytoplasmic granules in the cells of *H. cunea*, it was found that they average between 1 micron and 2 micra in diameter, or approximate the size of the nuclear bodies. The cytoplasmic granules tend to increase in size as they approach the lumen and finally, the droplets of secretion when passed into the cavity coalesce and become of considerable size (Fig. 16). Evidence of active secretion is correlated with an abundance of cytoplasmic bodies present in the cell, but not, as might be expected, with a decrease in the number of nuclear bodies present. It seems, therefore, that if the nucleus does play an active part in the formation of secretion that there is always an excess of nuclear bodies stored in the branched nucleus, and while the drain upon one part may be great, the reserve from another part of the nucleus may supply the demand.

The cytoplasmic bodies are found in all parts of the cell, but usually most abundantly between portions of a nucleus or between the nucleus and the lumen (Fig. 1). These bodies usually react to acid stains, but are generally stained black with iron hæmatoxylin. (See chart.)

That no cytoplasmic bodies are present in the middle section is difficult to determine with absolute certainty. If the nucleus with its nucleoloid bodies and nuclear bodies is responsible for secretion, surely secretion should occur in this region, for both types of bodies are present in the nuclei. Material fixed by the Gatenby method and stained with iron hæmatoxylin and acid fuchsin furnished the only indication that cytoplasmic bodies may be present in the reservoir region. The bodies took a light pink stain very similar to that of the clear, structureless substance

surrounding the silk core. These bodies were so indistinct that they may have been the result of poor technique.

Yamanouchi ('22) finds evidence that sericin, or the outer layer of the silk core, is secreted in the posterior portion of the middle region. Blanc ('89) considers that mucous is secreted in the anterior portion of the reservoir in the form of numerous granules, but this point has received little support in subsequent literature.

(*c*) *Mitochondria.*—Throughout the whole of the literature dealing with the silk glands of various lepidopteran larvæ, no reference has been found which even suggests that the workers suspected the presence of mitochondria in the cells. This may be due to the fact that no investigator has employed specific mitochondrial fixatives.

Maziarski ('11) describes certain bodies that are found occasionally in the protoplasm as having the form of short "bâtonnets," and staining deeply. These have a parallel arrangement along the shorter axis of the cell and unite with one another by fine protoplasmic fibrils. The substance of these bâtonnets is refractile and stains with acid stains. Gilson noticed these bodies and thought they were related to functional processes of the protoplasm in that they have the appearance of little tubules filled with secretory substance. Maziarski did not find these bodies consistently present in all of his material.

After fixation with Regaud, and staining with Altmann's anilin acid fuchsin, methyl green, clear-cut evidence of the existence of mitochondria in the cells of *H. cunea* has been obtained.

1. *Posterior Region.*—The mitochondria in the posterior region are very numerous, especially between portions of a nucleus and toward the lumen. In whatever part of the cell the mitochondria may appear, they always show a characteristic orientation with their longitudinal axis toward the lumen. There is no evidence that these bodies have any direct relation to the nucleus for they do not radiate from it or tend to cluster around it (Figs. 1, 2).

Their usual shape is filamentous, rod-shaped or granular. Where active secretion is taking place, long filaments are present,

averaging between 3 and 5 micra in length, and about 0.5 micron in diameter. In the more anterior portions of the gland, the mitochondria are found to be shorter, but equally numerous. Mitochondria do not appear to have any direct relation to the cytoplasmic bodies, but the latter are found situated among the mitochondrial filaments.

Material stained with iron hæmatoxylin after fixation in either the Champy or Gatenby method exhibits the presence of structures similar in size, shape and distribution to those observed with Regaud fixation (Figs. 5, 6).

2. *Middle Region.*—In the middle region, mitochondria are always present but their shape is somewhat different from that of the mitochondria in the posterior region. A gradual transition may be seen from the long filaments in the secreting region to the shorter rods in the anterior portion of this region (Fig. 7) and finally, in the true reservoir, the mitochondria become shortened to granules or spherules. The latter have the properties of mitochondria but are somewhat larger in diameter than the mitochondrial granules. It is not probable that the larger spherules represent secretion since in appearance, they more nearly resemble mitochondria than secretion bodies.

The distribution of mitochondria is fairly disperse throughout all parts of the cell, but with a tendency to be more abundant toward the lumen. No definite orientation can be distinguished in the longer rods (Fig. 12).

3. *Anterior Region.*—Bodies taking a stain characteristic of mitochondria are present in the anterior region, but these are always granular and arranged with little definite order (Figs. 8, 9).

4. *Critical.*—From the preceding observations, it is seen that mitochondria are present in the cytoplasm throughout the entire extent of the tubule. Their shape and to a less extent their number varies, however, in the three arbitrary divisions of the gland. Cowdry ('24, p. 318) suggests that mitochondria may serve to some extent as indicators of secretory polarity. In the case of silk secretion, it seems that this is truly the conclusion to be drawn. Secretion can be traced from the cell into the lumen, and mitochondria appear to be streaming toward the

lumen in a very definite way. There is no evidence that mitochondria actively produce secretion but only the indication that they may participate in the process. That mitochondria are present in the reservoir and conducting regions but that here they do not show polarity seems to indicate that mitochondria may be present to perform other functions than that of participating in secretory activity. Activity is occurring in both the middle and anterior regions, although not secretory activity. The middle portion appears to be a storage place for secretion where possible modifications of the secreted material may take place, for example, a condensation and compression. The secreted material then passes through the anterior conducting portion. Tanaka ('11) is the only investigator who has suggested any possibility of the existence of secretory function in the anterior region, and this probably only in the embryonic stages. The usual interpretation is that this region serves as a duct for the passage of secretion. In this case, the presence of mitochondria would seem to indicate that some activity exists within the cell, possibly of general metabolic nature.

(*d*) *Golgi Bodies.*—In preparations of glands fixed by the Mann-Kopsch, and by the Champy method, no structures have been found which could be interpreted as true Golgi bodies. In silk gland cells of *H. cunea* impregnated with osmic acid, numerous fine dark granules appear (Fig. 11), that are distributed very abundantly along the periphery of the cell-body and scattered more generally toward the lumen. These fine granules are present in all regions of the gland and always characteristically more abundant toward the periphery of the cell. In the extreme posterior tip of the gland, the granules appear grouped as a dense border which gradually becomes more disperse toward the lumen. In cells of the secreting region, groups of large, black bodies, spherical in shape and usually situated toward or near the lumen or the periphery are common and may be followed through several sections of a series.

Miss Murray ('26) describes a condition in the follicle cells of the cricket which seems quite similar and her figures compare closely with the condition found in the spinning-gland cell. With regard to her observations, she states (p. 223) that "In

the proximal region of some of these cells (follicle cells) there has been seen a nebulous structure composed of very finely divided black particles, which might be considered to indicate an excessive deposition of fat in the tissue. This fine emulsion might conceivably be coarsened at the boundary of the cell and coalesce into fatty globules."

In silk gland cells of *A. americana* and *D. virginica* which were fixed with DaFano's modification of the Cajal method, the same condition was found, but in these cases, there is a greater tendency toward an arrangement of the granules in rows, thus presenting the appearance of striations extending from the lumen toward the periphery, and likewise a greater grouping of granules around the edge of the cell toward the lumen. These bodies tend to concentrate along all sections of tracheæ, thus outlining the course of tracheids very definitely. In these two species, it must be remembered, active secretion of silk only visibly appears at the time of forming the cocoon.

It seems possible that these fine spherical granules may represent by products of cellular activity that are being eliminated. Their condensation along the tracheids and the proximal border of the cell may indicate that such may be the case. In the two species in which the process of secretion is only in the preparatory stage, an accumulation of waste products may be imagined to be occurring in the cell.

3. *The Membranes.*

(*a*) *Historical.*—Before the work of Helm ('76), the existence of a tunica propria, or external membrane, and a tunica intima, or internal membrane was known and had been described, but little was known concerning their structure or function. The tunica propria was quickly dismissed by Helm and those following him as a structureless membrane fitting very closely to the basal membrane throughout the entire extent of the gland that resists to a considerable degree the penetration of fixing fluids. But the tunica intima could not be so easily defined. Helm describes its structure in the anterior region as containing fine radial tubes which he refers to as "Porencanale." The entire intima, according to his observations, represents a cuticular

layer which is a modified secretion of the outer body wall and is, in consequence, shed at each moult. The fine canaliculi are arranged perpendicular to the surface and are interpreted to serve as conduction paths through the cuticular intima for the liquid secreted by the cells to the lumen. Blanc ('89) working on the silk gland cells of *Bombyx mori* concluded that the intima was a chitinous structure composed of very fine parallel threads encircling the lumen at intervals of from 3–4 micra and showing occasional anastomosis. Gilson ('94) confirmed the observations of Blanc. Maziarski ('11) describing the intima, pictures the structure as a thin membrane composed of a great number of filaments passing in spiral lines around the lumen of the canal and joined by fine transverse fibrillæ, thus producing the appearance of a network of threads. These filaments are described as staining deeply with iron hæmatoxylin and having the appearance of rigid fibrils located in the protoplasm of the cell nearest the lumen, and were thought to resemble organs of support but not to serve as passageways for substances elaborated in the cells.

Tanaka ('11) describes the intima as a striated, filamentous-appearing structure of chitinous nature, thickest in the anterior division and thin in the middle region. The filamentous striations appear to have a parallel arrangement except toward the posterior region where anastomosis may occur. This chitinous substance which gives rise to the intima is thought by Tanaka to be secreted in the anterior region and never to undergo ecdysis, but rather is added to in thickness with age. This is (p. 16), he concludes, "a fact which goes a long way in proving the secretion of this chitinous substance throughout the whole larval life."

Yamanouchi ('22) found the intima to be a glassy-like cuticular layer most conspicuously present in the anterior region where it has a thickness twice that in the other parts of the gland. This structure appears to be hyalin in nature, reacting to stains very similarly to sericin but more intensely in the anterior portion of the middle region, this structure is not homogeneous but gives the appearance of containing concentric bands whereby it is possible to distinguish two or three layers of intima. This arrangement in layers disappears anteriorly.

(*b*) *Observation.*—In the gland of *H. cunea* after fixation by the Regaud method, the presence of a clear, hyalin layer of material between lumen and cell-wall is found to be quite generally present in both the middle and anterior regions. (Figs. 7, 8, 9, 12.) This layer is always found to be very closely connected with the cell-wall, and yet to be perfectly distinct from the protoplasm. Oftentimes large vacuoles may be seen in the structure (Fig. 7), but these are always on the edge toward the cell and may be explained as the result of shrinkage at the time of fixation. This vacuolated condition occurs with greater frequency in the posterior portion of the middle region where the layer is thicker and less regular in form.

Posteriorly, in the secreting region, no indication of an intima is found except for the presence of a thin membrane along the inner edge of the cells. The cells usually have an irregular surface on the side toward the lumen, the protoplasm seeming to be in very close connection with the cavity (Fig. 2), but after fixation with Champy, Gatenby, or Benda preparations, this membrane is quite definite (Fig. 1).

Toward the more anterior portion of the middle region, fibrillæ may be seen extending through the clear hyalin substance and around the lumen. In longitudinal sections through the edge of the lumen, these structures appear to extend from the edge of the cell on one side of the lumen to the cell on the other side (Fig. 10), and to take their origin along the margin of the protoplasm. These fibrillæ stain darkly with iron hæmatoxylin and dark red with Altmann's acid fuchsin after fixation by the Champy method. In general, the fibrillæ tend to be arranged in parallel series and some evidence has been found of slight anastomosis between fibrillæ in the more posterior region. After the tube has become narrowed to form the conducting portion, fibrillæ disappear leaving the intima clear and structureless.

(*c*) *Critical.*—The fact that the intima is present in the anterior and middle portions of the gland as a thickened layer seems to indicate that this structure is not connected with active secretion but must serve some function related to conduction of secreted products to the outside. A copious supply of secretion is being poured continually into the reservoir from the posterior

region. To strengthen the cell against the resulting pressure and to prevent it from distortion, a thin layer of hyalin material might be conceived to serve as a support. At the point in the tube where it begins to narrow anteriorly, the pressure becomes greater and more support is necessary. That fibrillæ should appear in the transition region between the middle and anterior regions, is, therefore, to be expected. It is quite possible, moreover, that these fibrillæ may be more than merely supporting structures. Their shape and position around the lumen seems to indicate that they may have a contractile function, serving, perhaps, as a simple musculature in pushing the products of secretion into the long conducting tube. It is evident that some regulatory mechanism is necessary to prevent the small tube from becoming clogged by the ever accumulating secretion, and likewise, to regulate the amount of material passing through in order to produce a silk thread of uniform diameter.

4. *The Secretion.*

(*a*) *Historical.*—The morphological appearance of the secreted material has received considerably more attention throughout the literature than the mechanism of the secretion process, and yet little is definitely known about the real nature of the secreted substance. Staining reactions have been used almost exclusively as criteria upon which to base conclusions. Little detailed work has been done upon the actual chemical composition of the silk fiber further than a general analysis of the chemical components, the result of which has been to show that the fiber is made up of two slightly different substances; an outer, acidophilic layer of *sericin*, and a central core of basophilic *fibroin*. The former is quite similar in composition to the latter except that, according to Blanc ('89), it contains more oxygen, dissolves more readily in alkaline solutions and is more granular in appearance.

Tanaka ('11, p. 18–20) has reviewed very completely the results of previous workers and has arranged their views concerning the origin of fibroin and sericin into four principal groups. He then attempts to show that these theories have failed to satisfy all the conditions and proceeds to modify and combine parts of each theory into one more in accord with

his own conclusions which he states as follows: "The fibroin is produced by virtue of the physiological function of the gland cells, without reference to any part of the gland tube; the sericin is, in its formation from fibroin, quite indifferent from the physiological function of the cells; on the contrary this transformation goes on merely physico-chemically."

Maziarski ('11) in his enthusiasm for the nuclear origin of secretory products, states that the nucleoli which leave the nucleus become the fibroin and that chromatin, likewise, may be extruded into the protoplasm, there to undergo quite extended changes and finally pass out into the lumen as the sericin or gummy covering of the silk thread. This idea was not entirely new, for Vorhies ('08) had suggested that chromatin material may have a secretory function in addition to serving as the "bearer of heredity."

Nakahara ('11) recognizes only the formation of silk from transformed nucleoli, but Yamanouchi ('22) describes the production of sericin and fibroin in great detail and concludes that fibroin secretion takes place only in the posterior region, but that sericin secretion occurs in the posterior and middle parts of the middle region. The two kinds of secretion appear alternately and, due to the fact that each kind tends to remain separate, two layers are formed, distinguishable by their different staining reactions.

Blanc ('89) has described a third product of secretion, called by him "*mucoidine*," which envelops the silk fiber and stains more intensely with methyl green than either the fibroin or sericin. This substance, he thought, is formed in the anterior portion of the reservoir and may serve the function of a lubricant, thus facilitating the passage of the silk.

(*b*) *Observations*.—From a comparative study of silk gland cells prepared in different fixatives, it is evident that there exist in the lumen, substances which show different types of reactions to stains. After Regaud fixation, the silk thread appears to be perfectly homogeneous throughout its entire extent except in the anterior region of the gland where a slightly darker band may be distinguished around the thread. (Fig. 9.) The secretion found in the posterior and middle portions takes a reddish-blue stain

with Altmann's acid fuchsin, methyl green, and in the anterior portion, is decidedly green. The intima takes a bright red stain and appears to be a homogeneous, hyalin substance.

The condition of the secretion product after fixation with Champy, Benda, or Gatenby solutions is considerably different and leads to doubt concerning the identity of the various structures present in the lumen. With iron hæmatoxylin and acid fuchsin, the core of secretion is stained black. In the middle region, the secretion does not fill the lumen as is the case after fixation with Regaud, but between the secretion and the intima, now distinguished as a thin black membrane at the cell border, there is present a compact mass of finely granular substance that takes the acid stain. This substance, however, is stained light green in material fixed by the Champy, and stained by the Altmann method. Usually, the material appears to be homogeneous, but after Benda, and sometimes after Champy fixation, the substance is arranged in thread-like strands that extend from the cell-wall toward the secretion. That this band of substance is separate from the core of secretion is quite evident, for the secretion may be found entirely removed from the substance and be free in the lumen. The substance is never free from the edge of the gland cell. It would seem that either the amount of secretion present is determined by the width of this band which fills the remainder of the lumen or that the size of the band of substance is dependent upon the amount of secretion present, since after fixation, little or no shrinkage apparently occurs and the core of silk is contained in the center of the lumen closely surrounded by a band of this substance. The former hypothesis seems more probable since some regulatory mechanism for producing a thread of uniformly decreasing dimensions must be present.

Material fixed by the Champy, and stained with the Altmann method affords evidence of the presence of two substances in the thread. Secreted substance in the posterior portion of the gland stains light pink on the periphery while the center is either dark pink or green. This condition is not seen as sections are selected in the more anterior part of the gland. After fixation of the gland in Bouin, the silk thread exhibits two

distinct staining reactions. The core stains a clear pink and the periphery forms a black cortex around this core. This reaction of the secreted substance is consistent throughout the tube.

In silk glands of *D. virginica* fixed in Bouin and stained in iron hæmatoxylin and acid fuchsin, the silk thread has a central core of clear pink substance surrounded by a black peripheral cortex. A wide region filled with a reticulum or meshwork of fine pink granules surrounds the thread. Bordering this region and extending to the inner cell-wall, a wide compact band of fine granular substance arranged in concentric rings occurs. Throughout this band, numerous large drops of black substance appear which probably represent secretion material. By comparison of the condition just described, with that found in *H. cunea*, and also with conditions described by Yamanouchi, it appears that some difference exists between the type of gland which secretes continuously and that which secretes at definite periods only. The presence of two diverse staining reactions in the thread seems to indicate that two substances are consistently coexistent in the two different types of silk gland cell, but the exact relationship between these two substances has not been determined with certainty.

(*c*) *Critical.*—The question as to the identity of the substances within the lumen has presented a problem which cannot be definitely settled without further study both of preserved material from a variety of caterpillars and also of living material. Observation of the material at hand seems to show that in the posterior portion of the gland, the secreted substance is usually homogeneous, and this is likewise true in the middle region. But some modification of the secreted substance takes place in the anterior conducting region which gives the silk thread the appearance of being composed of two substances.

It is evident that even in forms which secrete silk during the entire larval life, the process is not a continuous one, for often within the lumen of the silk gland, the end of one thread can be seen and, close upon the posterior part of this thread, but separated from it by the substance filling the lumen, the end of another discrete cylinder of secretion begins (Fig. 7).

The substance which fills all available space in the lumen not occupied by silk secretion, may well be similar in its properties to mucus, lubricating the tube for the passage of the silk fiber. No origin for this substance has been found. It does not appear to be secreted by the cells of the gland, nor does it seem to be a dislodged part of the bodies of the cells.

Discussion.

1. *Relation of the Nucleus to Secretion.*

The nucleus and cytoplasm of the cells of the silk gland are closely associated in the production of the silk secretion. By many investigators, the nucleus has been considered the center of secretory activity in that the nucleoli migrate from the nucleus into the cytoplasm and there undergo modification. Study of the silk gland cells of *H. cunea* has given further indication of nuclear participation in silk production through the presence of nucleoloid bodies. These nucleoloid bodies do not themselves pass into the cytoplasm but appear to constrict pieces from their periphery that increase in size within the nucleus, finally passing through the nuclear wall into the cytoplasm where further modification of their substance occurs.

2. *Relation of Mitochondria to Secretion.*

Mitochondria having a characteristic arrangement in the cytoplasm of the secreting region are present in great numbers. It is quite possible that they take some part during the transitory stage when the secretion droplets are present in the cytoplasm, in modifying the material and transmitting it to the lumen. Mitochondria may act as catalysts, as suggested by Emberger ('25) or they may take a more direct part in secretion, but their presence and orientation toward the lumen seem to indicate that they are not passive cytoplasmic inclusions. Mitochondria are present throughout the extent of the gland but in the middle and anterior portions, they are shorter than in the secretory portion and show little definite orientation. In these portions, they may aid in the process of conduction and in general metabolic activity.

3. *The Nature of the Secretion.*

The column of silk secretion is apparently composed of two slightly different substances as evidenced by diverse staining reactions, but further study is necessary before the exact origin and nature of each is determined.

Since in the posterior region, the silk thread appears to be a homogeneous cylinder when preserved in several different fixatives, it would seem that there is but one kind of substance secreted and that this substance undergoes partial modification, possibly oxidation, during its progress down the tube.

A substance similar to mucous appears in the lumen as an envelop surrounding the silk column, but no account of its origin can be given. In life, it is evidently a fluid substance because it is found to be present wherever a break in the continuity of the fiber occurs. The distinction between this substance that is finely granular in appearance after certain fixations and the clear hyalin substance present in the same relative position, following fixation in Regaud's solution, cannot be determined. The latter substance has been considered as composing the intima but the former cannot be so accounted for.

Summary.

1. In the silk gland of *Hyphantria cunea*, three regions are distinguishable; a posterior, or secreting region; a middle region, or reservoir; and an anterior, or conducting region.

2. The nuclei of silk gland cells are greatly branched in the posterior region, but tend to be more flattened and less branched in the more anterior regions.

3. Three types of bodies are found to be present in each nucleus; the small basophilic granules, or chromatin; the intermediate acidophilic bodies generally termed nucleoli, but here called nuclear bodies; the relatively large, spherical body containing vacuoles and corresponding to a true nucleolus, hence called a "nucleoloid body."

4. The nucleoloid bodies appear to give rise to the nuclear bodies which pass out of the nucleus into the cytoplasm.

5. Cytoplasmic granules exhibit the same staining reactions as nuclear bodies and are approximately the same in size when they occur in the region of the nucleus.

6. Mitochondria are present in all parts of the gland, but only in the posterior region do they show definite orientation.

7. Two membranes are present along the course of the gland, and external tunica propria, and an internal tunica intima.

8. Two substances regularly appear to constitute the silk thread in the anterior region. This condition is found to continue more posteriorly after fixation with the Champy, Gatenby, Benda, and Bouin methods.

9. A substance similar to mucous surrounds the silk thread in the middle region but does not extend into the anterior portion. The origin of this substance has not been determined.

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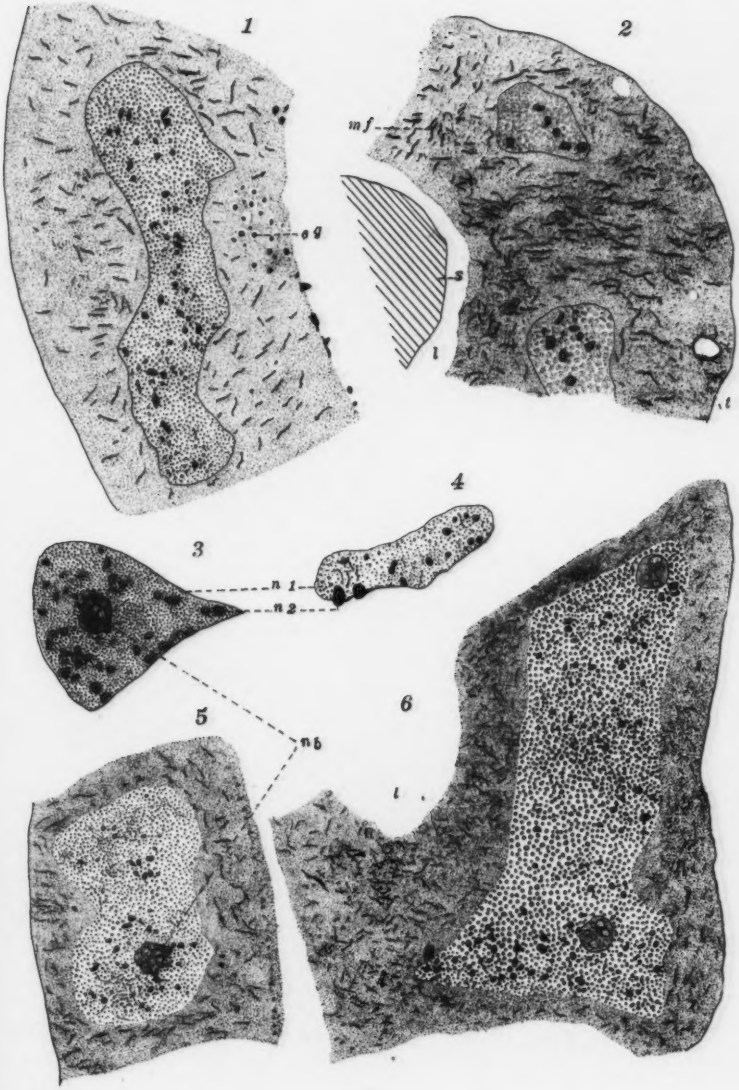
EXPLANATION OF PLATES.

PLATE I.

FIG. *I*. Diagrammatic outline of silk gland of *H. cunea*. Anterior, middle, and posterior regions of gland numbered 1, 2, and 3 respectively. Letters correspond to sections taken at these levels.

FIGS. *A–F*. Camera lucida outlines of sections from the three regions of a tubule fixed by the Regaud method. Cross sections cut 4 micra in thickness; magnification, oc. 10 ×, obj. 4 mm.

Note: Stippling represents cytoplasm; solid lines represent secretion; broken ines represent intima.



ELIZABETH KINNEY.

PLATE II.

All figures made from sections cut 4 micra in thickness, and drawn with camera lucida, magnification, oc. 12.5 X, obj. 1.8 mm., oil immersion.

FIG. 1. Section of cell from posterior region of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Cross section. Cytoplasmic granules appear to be grouping toward lumen.

FIG. 2. Section of cell from secreting region of tubule fixed by Regaud method and stained with acid fuchsin, methyl green. Cross section. Mitochondria show definite orientation toward lumen.

FIG. 3. A section of a nucleus from material fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. The nucleoloid body is surrounded by nuclear bodies that are smaller than those toward the nuclear wall.

FIG. 4. Section of nucleus from material fixed by the Gatenby method and stained with iron hæmatoxylin, acid fuchsin. Two nuclear bodies are in the process of passing through the nuclear wall into the cytoplasm.

FIG. 5. Longitudinal section of cell from posterior region of gland fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Nucleoloid body in process of constricting pieces from periphery.

FIG. 6. Longitudinal section of cell showing two nucleoloid bodies present in nucleus. Regaud fixation.

Symbols: *cg.* cytoplasmic granules; *l.* lumen; *mf.* mitochondrial filaments; *n1.* chromatin; *n2.* nuclear bodies; *nb.* nucleoloid body; *s.* secretion.

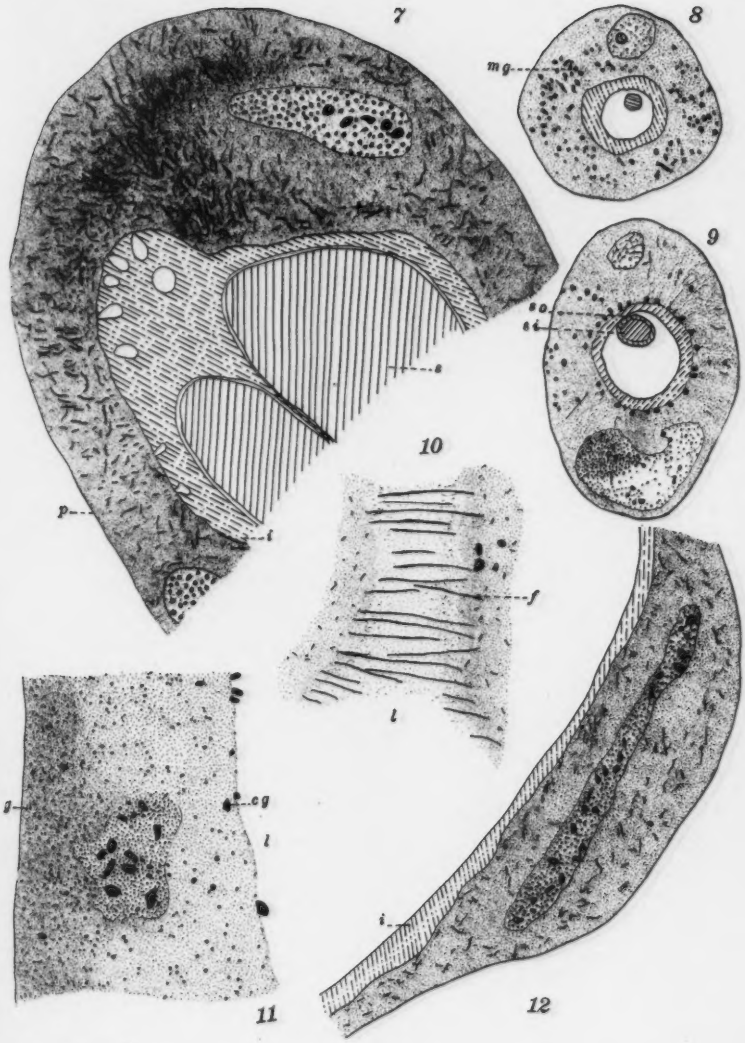


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PLATE III.

FIG. 7. Section of cell taken from posterior part of middle region of gland fixed by the Regaud method and stained with acid fuchsin, methyl green. Cross section. Hyalin layer appears irregular and contains vacuoles along the border toward cell. Mitochondria slightly shorter. Two columns of secretion chance to be present at this level.

FIG. 8. Cross section of tubule from anterior conducting region of gland fixed by the Regaud method and stained with acid fuchsin, methyl green. Mitochondria present as granules. Intima definite.

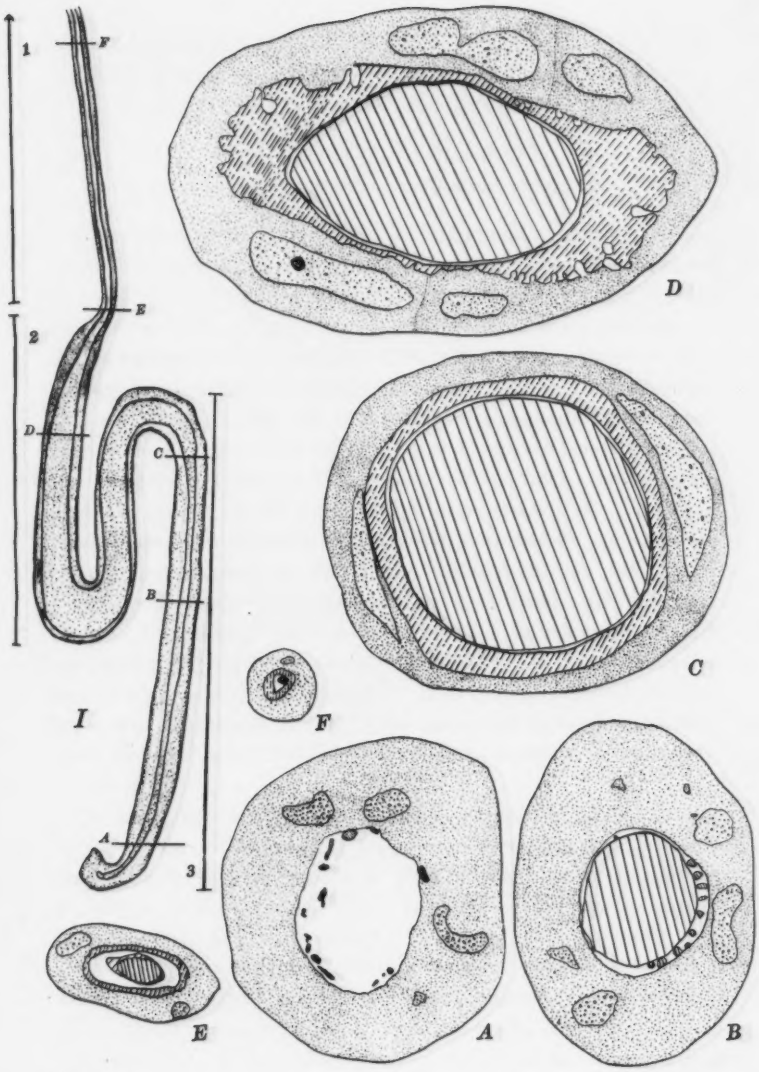
FIG. 9. Cross section from anterior conducting region of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Mitochondria appear as granules. Intima well defined, two types of secretion present.

FIG. 10. Longitudinal section of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Section taken from anterior part of the middle region at edge of lumen. Fibers appear extending from the edge of one cell to the edge of the cell opposite.

FIG. 11. Longitudinal section of cell from secreting region of tubule fixed by the Mann-Kopsch method, unstained. Numerous fine black granules fill cytoplasm and tend to concentrate toward the outer cell boundary.

FIG. 12. Cross section of cell from middle region of tubule fixed by the Regaud method, stained with acid fuchsin, methyl green. Mitochondria are shorter and less definitely orientated. Clear hyalin intima present along the edge toward lumen.

Symbols: f., fibers; *g.*, granules; *mg.*, mitochondrial granules; *p.*, tunica propria; *si.*, inner layer of secretion; *so.*, outer layer of secretion.



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THE LINKAGE DISTURBANCE INVOLVED IN THE CHROMOSOME TRANSLOCATION I. OF DROSOPHILA, AND ITS PROBABLE SIGNIFICANCE.

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The chromosomal abnormality in *Drosophila* known as "translocation I." was found by Bridges in 1917, and was first referred to by Morgan (1919); a preliminary account of it was given by Bridges in 1923. The case is a very remarkable one, unique in the literature to date, and it raises certain new problems concerning the arrangement which genes may have in a chromosome as well as concerning the changes in arrangement which they may undergo. The abnormality arose by a portion of the right hand end of chromosome II. breaking off and becoming attached near the middle of the right half of chromosome III. Bridges' data showed the size of the transposed piece and its approximate point of attachment on the third chromosome. It was known that the break in chromosome II. was between arc (ar) and plexus (pl), making the fragment about 8 units long, and that the transposed piece was attached somewhere between ebony (e) and rough (ro) in chromosome III. The deficient second chromosome was known as "Pale," from its effect on eosin-eyed flies, and the deficiency was lethal unless accompanied by translocation.

In the hope of getting further light on the chromosome change involved, the writer undertook to test more extensively the third chromosome linkage values in flies which carried the translocation. I wish to take this opportunity to express my indebtedness to Dr. H. J. Muller, who suggested the work, and whose aid and suggestions have made it possible. In these experiments the homozygous as well as the heterozygous condition of translocation was studied, and all of the left hand end of Chromosome III. was under genetic observation. Bridges had already shown that crossing over was greatly reduced in the region close to the locus of translocation; but his data included

only flies heterozygous for the transposed piece. The comparison of the homozygous and the heterozygous conditions with each other is of especial interest because of its bearing on the problem of the mode of attachment of the second chromosome fragment, as will be shown later.

The third chromosome mutant genes used in the experiments are, in their order from left to right along the chromosome: roughoid (ru), hairy (h), scarlet (st), pink (p), spineless (ss), delta (Δ), hairless (H), ebony (e), rough (ro), and claret (ca). Their locations are shown in the map of Fig. 1. Translocation is represented by Tr, and the deficient second chromosome by Pl. Curly (Cy) is a second chromosome gene used in balancing stocks. The linkage in flies homozygous for Tr was tested in two experiments. The summarized results for these crosses are given below, in Tables I. and II.

TABLE I.

$\frac{Cy}{Pl}$ $\frac{\Delta\ H\ e\ Tr}{ru\ h\ st\ p\ ss \qquad Tr}$ ♀ × ru h st p ss e ♂.

Regional Crossover Frequencies.						Total.
ru–h.	h–st.	st–p.	p–ss.	ss–Δ.	Δ–H.	
285	202	45	86	61	10	1,097
26.0%	18.4%	4.1%	7.8%	5.6%	.9%	

TABLE II.

$\frac{Cy}{Pl}$ $\frac{\Delta\ H\ e\ Tr}{Tr\ ro\ ca}$ ♀ × e ro ca ♂.

Regional Crossover Frequencies.				Total.
Δ–H.	H–e.	e–ro.	ro–ca.	
8	13	27	16	463
1.7%	2.8%	5.9%	3.5%	

These figures show a remarkable drop below normal in the crossover values as the locus of translocation is approached. This may be seen by comparing their map values (see Fig. 1,

b and *c*) with the normal third chromosome map (Fig. 1, *a*). The map length of the left-hand third will be seen to be slightly increased, but the genes between pink and claret are crowded much closer together. There is an apparent lengthening of the

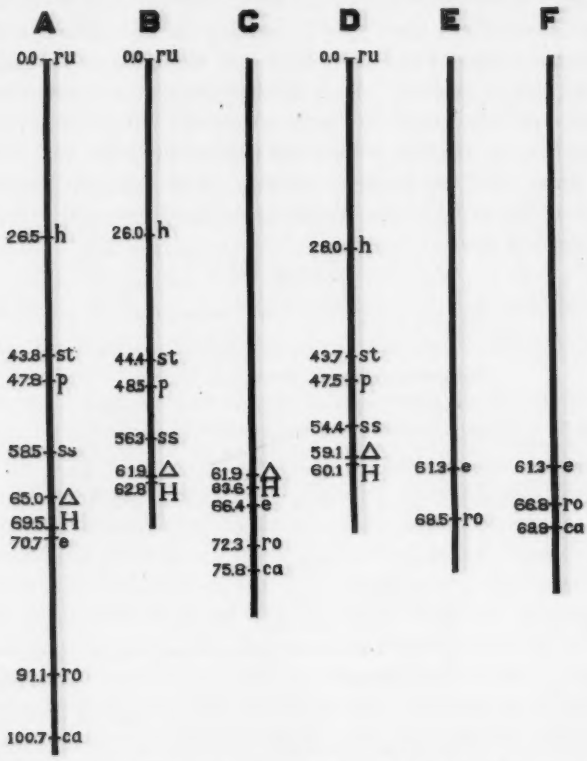


FIG. 1. (*a*) The normal third chromosome map. (*b*) and (*c*) Maps of third chromosome homozygous for translocation. (*d*), (*e*) and (*f*) Maps of third chromosome heterozygous for translocation.

distance between hairless and ebony, but this distance is so short, and the probable error so large, that the increase is probably not significant. Between ebony and rough, the region where the second chromosome fragment is attached, the per cent. of crossing over is less than one third of the normal value. These

relations are shown in Fig. 2, *a*, which shows the ratios of the values obtained in these experiments to the standard values of each of the major regions studied.

At the same time that these crosses were being made, the linkage in flies heterozygous for the abnormality was being tested. The same stocks were used in making up the crosses, and the characters used were introduced into the cross in as near the same way as possible. Both the homozygous and heterozygous lines were kept under the same conditions, so that any possible variations in viability would affect both lines alike and allow us to draw valid comparisons between them. As will be seen in Tables III. and IV., the results were almost identical with those of the first tests.

TABLE III.

$$\frac{}{\text{Pl}}\ \frac{\Delta\ \text{H e Tr}}{\text{ru h st p ss}\quad\ \text{e}}\ \text{♀} \times \text{ru h st p ss e ♂}.$$

Regional Crossover Frequencies.						Total.
ru–h.	h–st.	st–p.	p–ss.	ss–Δ.	Δ–H.	
162	91	22	40	27	6	578
28.0%	15.7%	3.8%	6.9%	4.7%	1.0%	

TABLE IV.

$$\frac{}{\text{Pl}}\ \frac{\text{ru h st p ss}\quad\ \text{Tr}}{\text{p ss e}\quad\ \text{ro}}\ \text{♀} \times \text{p ss e ro ♂}.$$

Crossovers.		Total Flies.
(ru h st) p ss e	(ru h st) p ss ro	
42	41	
Total Crossovers 83		1,155
7.2%		

In Table V. are results for a test involving the ebony-rough and rough-claret distances. Only those flies which did not show delta hairless could be used in computing crossover values in this experiment. This cross also gave data on the exact location of translocation, as will be discussed later.

TABLE V.

$$\frac{Cy\ ru\ h\ st\ p\ ss\quad Tr}{e\quad ro\ ca}\ \text{♀} \times \frac{Cy\ \Delta\ H\ e\ Tr}{Pl\quad e\quad ro\ ca}\ \text{♂}.$$

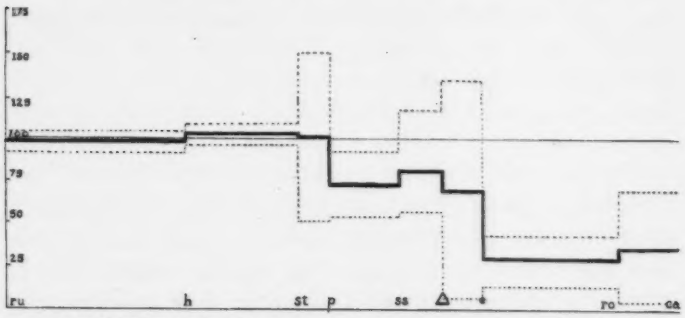
Regional Crossover Frequencies.		Total Utilizable Count.
e–ro.	ro–ca.	
50	28	902
5.5%	3.1%	

The results given in the last three tables are summed up in the chromosome maps shown in Fig. 1, *d*, *e*, and *f*, and Fig. 2, *b* shows the ratios of these values to the standard values. It will be seen on comparing these results with those based on the homozygous Tr flies that there is no noticeable difference in the crossover effects produced by the homozygous and by the heterozygous conditions.

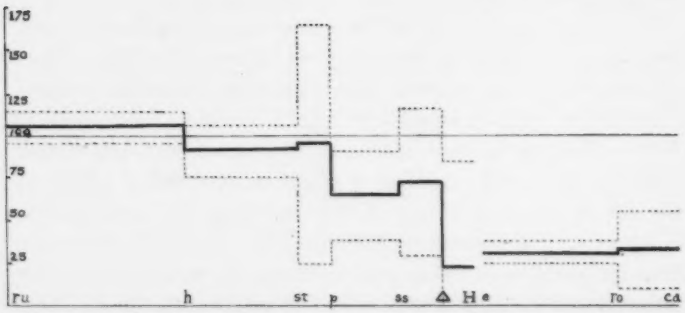
The chief significance of the results probably lies in their bearing on the mode of attachment of the translocation. There are two conceivable ways in which the translocated piece might be attached between ebony and rough: it might be interpolated within this length, thus causing a prolongation of the thread between ebony and rough, or it might be attached somehow to the side of the original thread, thus resulting in a configuration of genes and of chromatin different from what had previously been regarded as normal, and not entirely in one line. The present results clearly disprove the first alternative, for if it were true, crossing over between ebony and rough should be increased when the flies were homozygous for the interpolated fragment. We must therefore conclude that the chromosome carrying translocation is branched or doubled in the region where the latter is attached.

The second point of significance is that the presence of translocation does not reduce crossing over throughout the chromosome. Beginning at the left-hand end of the chromosome, we find that the number of crossovers is practically normal until we reach the locus of pink, one third of the distance along the chromosome. From this point on, the map distance is markedly

shortened until the locus of translocation is reached. In this interval, between ebony and rough, the per cent. of crossing over is less than one third that of the normal. Between rough and



HOMOZYGOUS TRANSLOCATION



HETEROZYGOUS TRANSLOCATION

FIG. 2. Chart showing the ratios (in percentages) of the observed crossover values to the standard. The abscissa represents the length of the III. chromosome from roughoid to claret; the ordinate represents the percentage which the observed crossovers form of the standard value in each region. The heavy line represents the percentage observed; the dotted lines mark the limits of the probable error of this percentage, calculated for each interval.

claret the amount of crossing over rises again, approaching half the normal value. The number of flies involved was not large enough to yield reliable data on the coincidences, but as far as they went they agreed well with the normal.

If crossing over takes place at a stage in synapsis in which the chromosomal threads are twisted about each other, we can readily see why the presence of the attached fragment in either one or both of the third chromosomes should produce the effects that it does. Either by stiffening the threads so that they could not twist as tightly as in the rest of the chromosome, or by an actual interference with the loops, the transposed piece should tend to prevent the close association between the homologous chromosomes necessary for that interchange of genes which constitutes crossing over. This interference would produce its maximum effect at the point of attachment, and the effect would decrease to either side. This gradual decrease in the interference is well shown to the left of ebony. The locus of translocation is too close to the right-hand end of the chromosome to show any marked diminution of its effect toward that side. The rough-claret crossovers are, however, nearer to the normal percentage than those in the ebony-rough interval.

In the cross shown in Table V., 36 crossovers between ebony and rough were observed in which it was possible to follow the behavior of the translocation. The results are shown below.

TABLE VI.

$$\frac{Cy}{\quad}\ \frac{ru\ h\ st\ p\ ss\quad Tr}{e\qquad ro\ ca}\ \text{♀} \times \frac{Cy}{Pl}\ \frac{\Delta\ H\ e\ Tr}{e\qquad ro\ ca}\ \text{♂}.$$

Crossovers between (e) and (Tr) $\frac{\quad}{Pl}\ \frac{e\ Tr}{e\quad ro\ ca}$	Crossovers between (Tr) and (ro) $\frac{\quad}{Pl}\ \frac{(ru\ h\ st\ p\ ss)\quad Tr\ ro\ (ca)}{\quad .\quad e\quad ro\ ca}$
11	25
36	

Translocation is, according to these figures, located at approximately 11/36 of the distance between ebony and rough, or at 76.9 on the chromosome map. The data given in Bridges and Morgan's monograph on the III. chromosome characters also show translocation to be between ebony and rough, but their figures show the locus of translocation to be closer to rough than to ebony. Also, the amount of crossing over in the ebony-

rough interval is even lower than in the experiments here reported. These differences may be due to a differential viability in the two cases; there may also be differences in genes influencing crossing over, but such differences in ratios do not affect the principal conclusion of this paper. The important point is that the effect of the translocation is the same whether it is heterozygous or homozygous.

SUMMARY.

1. The chromosomal abnormality in *Drosophila*, known as translocation I., is located at approximately 76.9 on the third chromosome.

2. The presence of translocation does not affect the crossover values for the first 45 units in the left-hand end of the chromosome. To the right of this point, the per cent. of crossing over progressively decreases as the locus of translocation is approached, reaching its minimum of less than one third normal value in the region between ebony and rough.

3. The same results are obtained no matter whether the translocation is homozygous or heterozygous.

4. The fact that the results are the same in both heterozygous and homozygous flies shows that the attached fragment is not interpolated in the third chromosome, but is attached to its side. The resulting chromosome is thus of a type which has hitherto not been reported: that is, one in which the genes are not in a single linear series.

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THE CHROMOSOMES OF THE CHICK SOMA.

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The accumulated evidence in favor of the chromosomes being in some way concerned with the determination and control of the somatic characters has become so impressive as to be almost unassailable despite the possible objection of a few that the last link in the chain (a demonstration of the actual activity of these nuclear bodies as hereditary character bearers), due to the deficiencies in our knowledge of cell chemistry, has yet to be forged. This latter point is one about which geneticists and cytologists are, at present, not greatly concerned and conclusive data upon the physiological behavior of the chromosomes does not seem at all imminent. While information is gradually accumulating in preparation for this final analysis much may still be accomplished by purely morphological studies with tentative interpretations of physiological activity based on these observations. A recent study of the chromosomes associated with sex in the chick (5) involved extensive observations on the chromosomes of dividing somatic cells, the results of which are recorded below.

OBSERVATIONS.

The technique of preparation (3), of counting and measuring the chromosomes, the amount and source of the material (5) have all been recorded and need not be repeated here. The chromosomes of over 150 cells have been drawn and studied. As the nervous system is the most actively growing of all the regions of the body the majority of the cells studied have naturally been found in some part of it. Cells with clear polar views of metaphase chromosomes have been found in the brain, neural tube,

[1] The observations recorded in this paper were made principally at the University of Pennsylvania during the writer's tenure of a National Research Council Fellowship.

optic cup, auditory vesicle, connective tissue, heart muscle, blood, amnion, gonads and in tissue cultures of muscles.

The Chromosome Number.—The difficulties involved in determining the exact number of chromosomes in the chick have been described in detail (5). Briefly, the trouble encountered may be said to be due, first to the extreme smallness of the shortest chromosomes of the complex making observation at times uncertain and second, to the apparent failure of the component granules of the smaller chromosomes to unite or at least to clearly indicate their proper relations to each other until late metaphase if indeed it really and always happens then.

The average number of chromosomes in the soma of the chick, based on 78 counts, is 33. I think that this is perhaps lower than the actual number which the most satisfactory counts indicate to be about 35 or 36. This variation in the chick is apparently due to a failure of the chromomeres or parts of the smaller chromosomes to unite rather than to fragmentation as in the case of the pig (1). This opinion is based upon the observations in the chick of larger numbers of distinct chromatin bodies (with occasional visible connecting threads between them) in the prophase than could be found in cells in later stages of mitosis whereas in the pig a reduction in the chromosome number as the metaphase was neared did not occur. The smaller chromosomes of the chick are the only ones concerned while in the pig the long ones are the ones that become broken up.

The Chromosome Form.—All of the longer chromosomes (about 12 in number) of the somatic cells are in the form of J's while the shorter ones are rods. Their structure and size relations (5) are alike in all the tissues studied including tissue cultures (4). The largest chromosome (chromosome pair in the male) of the complex has been shown to be the one associated with sex and is found in all somatic cells as clearly as in the cells of the gonads (5), Figs. 1 to 9. The female embryonic cells are heterozygous for this chromosome while the male cells are homozygous. No differences in the chromosomes or their behavior have been noted between any of the body tissues or in comparison with those of the embryonic germ cells.

Discussion.

The morphological data on the behavior of the chromosomes in the developing embryo, although admittedly scant, has so far given no clue to the manner in which the chromosomes may contribute their potentialities to the growing organism. Before a study of somatic chromosomes had been made it seemed reasonable to expect to find the various highly differentiated cells of the body with chromosome numbers, morphology or behavior at variance both with those found in other tissues and with the specific number and general characteristics found in the gonads. This has been found not to be the case in at least three forms, the pig (1), the evening primrose (2) and the chick (5). There was some fragmentation of the somatic chromosomes in the first two forms but it was shown that none of the chromatin was lost. Furthermore this fragmentation was neither specific for any particular tissue nor constant in amount. In general the chromosome situation in the soma seems to be entirely similar to that found in the unreduced gonad cells. This is a matter difficult to understand in the present state of our information. Of all the characteristics that must be controlled or borne by or at least associated with the chromosomes (as shown by the studies on *Drosophila*) few have any chance for expression in the majority of the somatic cells and tissues and must therefore be inhibited in one way or another. The cells of the lower forms of animal life largely retain the germ-like power of reproducing the portion lost or even an entire organism following injury. This power is perhaps even more marked in plants. As differentiation becomes more extreme in the animal kingdom the ability to regenerate a part or a whole animal from cells already specialized becomes less and less until in the highest types of animals somatic cells are usually able to produce only somatic cells like themselves. Yet the chromosomes in these highly specialized cells have, in the examples studied, been found to be entirely similar to those in the germ cells containing the possibilities for a complex animal or plant. This may suggest that the chromosomes are functionless in the differentiated Soma. Or it may be that having contributed their share in the production of the specialized tissue are thereafter inactive as far as are concerned the general somatic

attributes or determiners with which originally they must have been equipped. If the latter interpretation is correct the perpetuation of the complete mitotic mechanism in the soma as it exists in the reproductive organs may seem, as far as any need for an exact division of the genes is concerned, somewhat unnecessary, to be classed possibly with vestigial organs and having no more significance. In view of the great delicacy of the mechanism that exists in all cells for accurately dividing the chromatin the last suggestion does not seem impressive. But as far as our morphological data on the behavior of somatic chromosomes go, together with the behavior of the soma in growth and regeneration, the above suggestion is at least a possibility to be considered in our attempt to get at the physiology of development and genetics.

SUMMARY.

1. The chromosome number in the somatic cells of the chick is about 35 or 36.

2. No characteristic differences between the number, the sizes, the morphology or the behavior of the chromosomes in comparison either with each other or with the cells of the gonads have been noted.

3. In view of the entire similarity of the somatic and germinal mitotic behavior and in consideration of the complete inability of highly specialized cells to regenerate other than cells similar to themselves it is tentatively suggested as a basis for future discussion that the somatic chromosomes, as far at least as their genetic function is concerned, have either become functionless or their cytoplasmic environment is incapable of reacting to the possibilities presumably carried by them.

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DESCRIPTION OF PLATE I.

All figures were drawn at table level with a Zeiss 1.5 mm. apochromat and a 15 × Orthoscopic ocular. They are reproduced at the size originally drawn. All are polar views of metaphase plates in chick embryonic cells with the exception of Fig. 5.

FIG. 1. From optic cup. 35 chromosomes.

FIG. 2. From amnion. 30 chromosomes.

FIG. 3. From a male gonad. 39 chromosomes.

FIG. 4. From neural tube. 32 chromosomes.

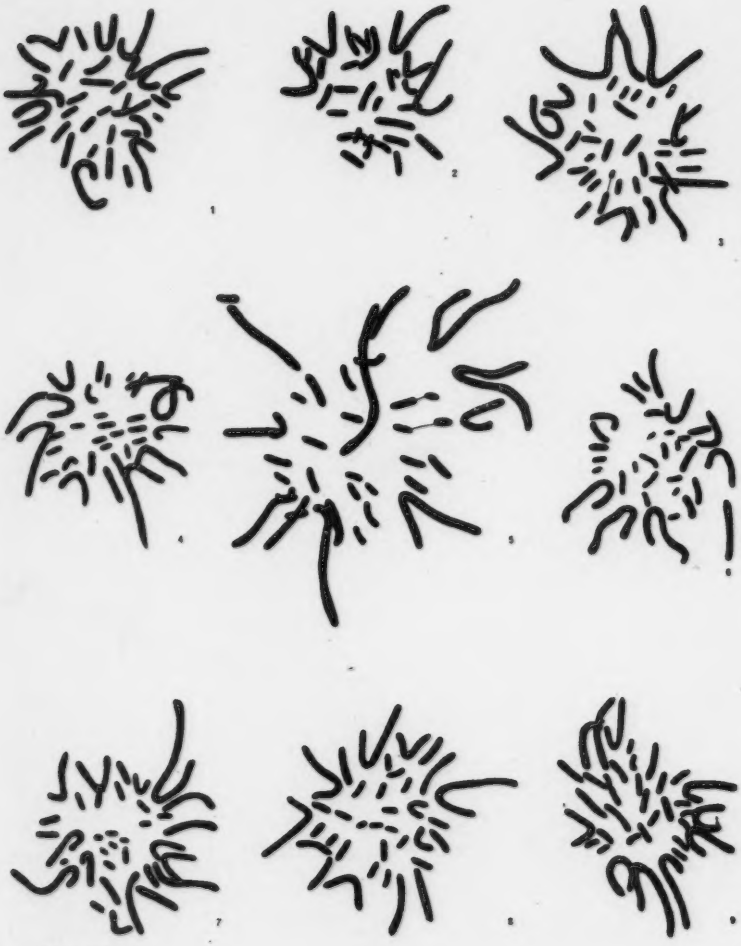
FIG. 5. Late prophase in connective tissue cell grown in a tissue culture. 38 chromosomes.

FIG. 6. From heart muscle. 33 chromosomes.

FIG. 7. From brain. 35 chromosomes.

FIG. 8. From female gonad. 36 chromosomes.

FIG. 9. From neural tube. 35 chromosomes.



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